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X-RAY DIFFRACTION PATTERNS OF SONICALLY DISRUPTED MICROORGANISMS

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SUMMARY. When suitable prepared extracts of microbial cells are examined by monochromatic x-rays in the x-ray diffractometer both qualitative and quantitative differences may be noted in the diffractograms obtained with extracts of different species and strains of microorganisms. Many of these diffraction patterns have been shown to have peaks that are nearly identical, and which are contributed by halite and sylvite. Removal of halide salts by electrodialysis makes it possible to demonstrate the presence of weaker diffractive crystallites in dried cell extracts. The usefulness of this technique for cytochemical studies and for taxonomic purposes is discussed.

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X-rays have been used for the examination of biological materials for almost 40 years (Scherrer, 1930; Herzog and Jancke, 1920) and have proved to be very useful in studies of the fine structure of homogeneous preparations of macromolecules (Astbury, et al., 1948; Franklin, 1956, Bear, 1957; Wilkins, et al., 1959; and Engstrom and Finean, 1958). Fibrous, myelin, and crystalline forms of pure chemical species have given intense diffraction with soft x-rays, and after 'isomorphic' modification of globular proteins the unit cell of this type of protein may also be elucidated (Crick and Magdoff, 1956).

For the most part, little information is gained from x-ray diffraction studies of normal intact tissue cells: the cellulose pattern may be recognized in the maturing plant cell wall; crystalline inclusions in some protozoan cells give a characteristic diffraction pattern; but other cell components merely contribute to a very high background scattering, whether the sample is examined in the wet or dry state. As exceptions, cells with an extremely high concentration of highly polymerized desoxyribonucleic acid present in well-oriented arrangement, such as sperm and plant virus cells, give recognizable x-ray diffraction patterns; calciferous cells also have been shown to give distinctive x-ray diffraction patterns (Spiegel-Adolf and Henny, 1947).

However, when tissue cells are disrupted by appropriate means, and the cell solutes are separated and dried, they are capable of producing

distinctive x-ray diffraction patterns, typical of well-crystallized compounds. A preliminary study of this technique is presented in this paper.

MATERIALS AND METHODS

Cultures:

1. Staphylococcus aureus No. 4 VDL (Iowa State University Veterinary Diagnostic Laboratory). Slightly hemolytic and strongly coagulase positive strain. From skin lesions of a dog.

2. Staphylococcus aureus No. 10 VDL. Strongly hemolytic and strongly coagulase positive strain. Original source unknown.

3. Staphylococcus aureus 3A5. Nonhemolytic and coagulase negative strain. Carried in departmental stock culture collection since 1954. Original source unknown.

4. Staphylococcus albus 3A8. Nonhemolytic and coagulase negative strain. Carried in departmental stock culture collection since 1949. (ATCC 151)

5. Micrococcus flavus 3A34. Nonhemolytic and coagulase negative strain. Carried in departmental stock culture collection since 1958. (ATCC 10240)

6. Escherichia coli K-12 and Escherichia coli K-12/N5. From the collection of Dr. William R. Lockhart, Iowa State University, Department of Bacteriology.

Media and Solutions:

1. Staphylococcus medium No. 110 (Difco)

2. Escherichia coli medium A (Davis and Mingioli, 1950) modified:

Solution A:	$K_2HPO_4 \cdot 3H_2O$	7.0 g	$MgSO_4 \cdot 7H_2O$	0.1 g
	KH_2PO_4	2.0 g	$(NH_4)_2SO_4$	1.0 g
	Na Citrate $\cdot 5H_2O$	0.5 g		

Deionized water to 250 ml. pH adjusted to 7.0 with 1 N KOH before autoclaving at 15 lbs steam pressure for 20 minutes.

Solution B: Glucose 30 g
Deionized water to 250 ml volume. Autoclave at 15 lbs steam pressure for 20 minutes without adjustment of pH.

Solution C: Noble agar (Difco) 15 g
Deionized water to 500 ml. pH adjusted to 7.0 with 1 N KOH before autoclaving. For agar medium, the indicated volumes of solutions A, B, and C were combined at 45°C to give 1 liter of medium A, modified.

When liquid medium was desired, Solutions A and B were each made with 500 ml of deionized water. All glassware used with this synthetic medium was soaked in C and M solution, 1:100, then rinsed with several changes of deionized water.

3. Bhitsye medium:	Brain Heart Infusion (Difco)	37 g
	Trypticase Soy Broth (BBL)	30 g
	Bacto-Yeast Extract	10 g

Deionized water to 1000 ml. pH adjusted to 7.0 with 1 N NaOH before autoclaving at 15 lbs steam pressure for 20 minutes.

When semisolid medium was desired, 1.5% Noble agar was added.

4. C and M Solution:	Calgon*	42.3 g
	Sodium metasilicate	380.5 g
	Distilled water to 4000 ml.	

Cultivation of Cultures

In earlier work, the colonial growth of test microorganisms was produced on large agar culture dishes (15 x 8 x 2 inches Pyrex baking dishes) which were covered with heavy aluminum foil during incubation. Each agar dish was inoculated with 5 ml of 24-hour broth culture of the appropriate microorganisms, and the inoculum was distributed evenly by addition of a few sterile glass beads and repeated tilting of the dish. Plates were incubated in normal position for 24 hours at 37°C.

More recently, the 15-liter New Brunswick fermenter has been used to facilitate mass culture of the test microorganisms, using the same media, without agar, that were used in the agar plate culture method. Here, 5-10% inoculation rates with a 24-hour stationary broth culture of the appropriate test microorganism were used. Aeration rates were 0.5 liter of air per minute per liter of medium initially, and 0.3 liter of air per minute per liter of medium after foaming developed. Stirring speed was 160 rpm throughout the 36-hour incubation period, with the temperature held at 37°C.

Cell Harvesting Methods

1. With agar grown cells, the growth was scraped from the agar with the minimum volume of physiological saline needed to remove the cells efficiently, using a bent glass stirring rod as a scraper. Glass beads carried over from the inoculation step, and bits of agar were filtered out with quadruple thickness surgical gauze, and the heavy cell suspension was collected in centrifuge tubes for the washing operation.

2. Cells from broth cultures were collected in a Servall Model SS-3 Superspeed Centrifuge at 10,000 rpm or in a Sharples centrifuge at 20,000 rpm, and the packed cells were resuspended in 10 volumes of 0.85% sodium chloride solution. About 50 gm of cell paste were obtained from 10 liters of culture medium. This heavy cell suspension was transferred to centrifuge tubes for washing.

* Calgon Inc., Pittsburgh, Pa.

Cell Washing Procedure

1. Agar grown cells were spun down in the Servall Superspeed Centrifuge at 10,000 rpm for 10 minutes, then resuspended in 10 volumes of normal saline solution. The supernatant liquid was decanted and discarded, following a second centrifugation at 10,000 for 10 minutes.

2. With cells grown in broth culture, the saline suspension was centrifuged at 10,000 rpm for 10 minutes, and the supernatant liquid was decanted and discarded.

Preparation of Cell-Free Extracts

1. Washed cells were resuspended in deionized water, using 10 volumes of deionized water for each volume of packed cells. Cells were disrupted by treatment in the 10 kilocycle Raytheon Sonic Oscillator for 10-20 minutes, with the time dependent on the amount of treatment required to cause a definite reduction in the number of intact cells, as determined by microscopic examination of a simple stain of the cells.

2. Intact cells and debris from the sonic treatment were removed by centrifugation at 10,000 rpm for 10 minutes, and the supernatant cell-free extract was dried in a National anaerobic incubator* at 29 inches of vacuum or better. For some preparations, such as samples of medium with high glucose content, temperatures below 30°C were required to prevent carmelization during drying, but for low sugar preparations, a temperature of 70°C was applied to shorten the drying period. Routinely, 50 ml aliquots of cell extracts dried overnight when the sample was spread as a thin film in a large Pyrex baking dish. The dried film was scraped from the glass with a single-edge razor blade, and was ground to approximately 200 mesh in a mortar and pestle to insure homogeneity of grain size before it was examined in the x-ray diffractometer.

X-Ray Diffraction Analysis

1. The dried cell extract was mounted in a suitable metal sample holder which was then positioned in the Norelco x-ray diffractometer. The x-ray tube was operated at 35 KV and 15 ma. Colimating slits routinely used were: divergent slit=1°, scatter slit=1°, and receiving slit=0.006 inch. The scanning speed was normally 1° per minute, chart speed 1/2 inch per minute, scale factor=4, and time constant=2.

2. Preferred orientation of crystalline materials was reduced by use of a vibrating filling funnel when loading the sample holders.

RESULTS

Using the above procedures, approximately 50 x-ray diffraction patterns of dried cell extracts were obtained. A comparison of these diffractograms, checking the recorder pattern at each 0.05° Bragg angle, revealed an abundance of peaks which were often distinctive in (a) location, (b) shape, and (c) amplitude. For presentation purposes, the principal diffraction peaks given by dried extracts of five different species of bacteria were assembled in bar graph form, shown in Table 1.

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* National Appliance Company, Portland, Oregon.

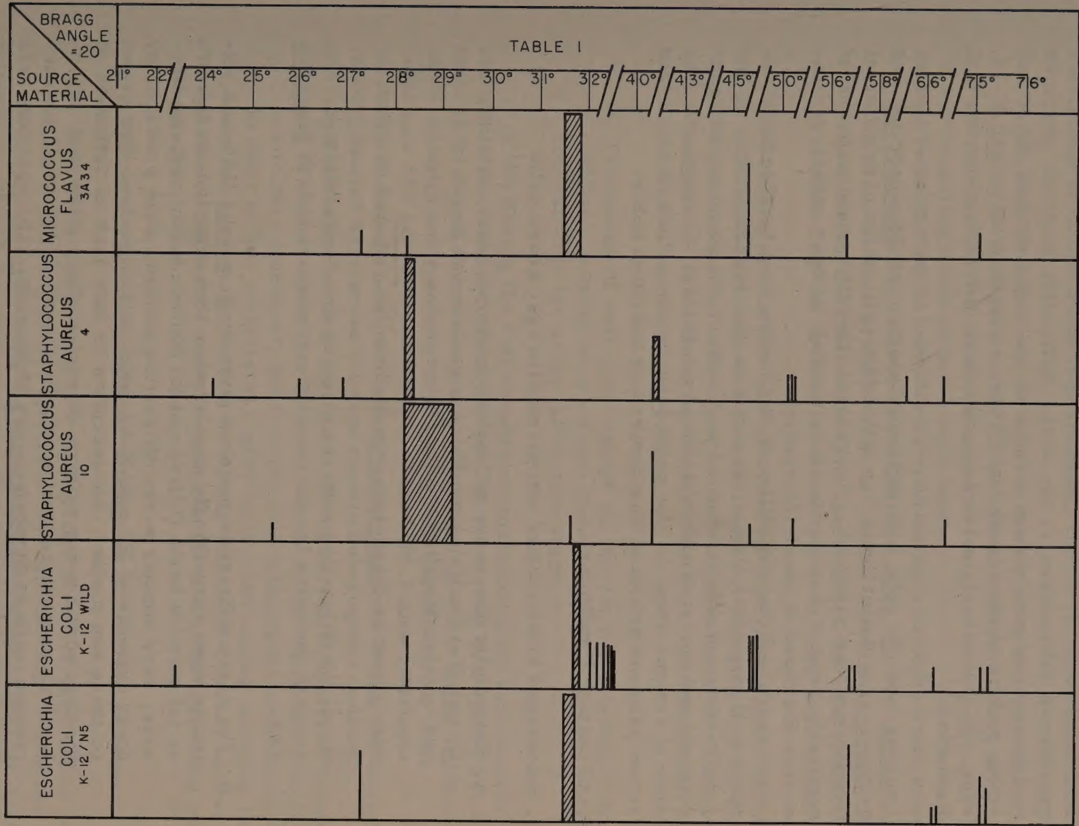
TABLE I
BRAGG ANGLE = 2θ
SOURCE MATERIAL
21° 22° 24° 25° 26° 27° 28° 29° 30° 31° 32° 40° 43° 45° 50° 56° 58° 66° 75° 76°
MICROCOCCUS FLAVUS 3A34
STAPHYLOCOCCUS AUREUS 4
STAPHYLOCOCCUS AUREUS 10
ESCHERICHIA COLI K-12 WILD
ESCHERICHIA COLI K-12 / N5

Each of the different microorganisms compared in Table 1 was grown in Bhitsye fluid medium, and extracts from them were prepared under standard conditions. It is quite apparent that there are both qualitative and quantitative differences in the x-ray diffraction patterns produced by dried extracts of even mutant strains of an organism and the x-ray diffraction pattern of the parent 'wild' type, as typified by E. coli K-12. Likewise, the less closely related species show diffraction patterns that are distinctive.

When this method of examination was extended to include other strains of S. aureus and E. coli, and different species of Micrococcus and Staphylococcus, a distinctive x-ray diffraction pattern for each different microorganism was produced. In general, the diffraction patterns of taxonomically related microorganisms seemed to bear closer resemblance than did those of unrelated species.

It was recognized that the utility of this finding as a tool for the characterization of microbial tissue depended on the reproducibility of the x-ray diffraction patterns produced by an extract of a given microorganism obtained and analyzed under standard conditions. Consequently, the influence of various steps in the procedure on the nature of the x-ray diffraction patterns produced was checked as indicated below:

Effect of Culture Conditions

1. Submerged broth culture compared with agar grown cells:

   A. Essentially the same diffraction patterns were obtained for E. coli K-12 cells whether this organism was grown in aerated and agitated Modified Medium A, or on the same medium made semisolid with 1.5% Noble agar; when S. aureus 3A5 was likewise grown on Staphylococcus Medium No.110, and on a similar medium compounded without agar for submerged culture growth, the x-ray diffraction patterns given by dried extracts were essentially the same for the two different preparations of S. aureus 3A5.

   B. The x-ray diffraction patterns given by S. aureus 3A5 cell extracts were surprisingly similar when this organism was grown on (a) nutrient broth (Difco) and (b) Bhitsye broth medium; likewise, very similar x-ray diffraction patterns were produced by dried extracts of E. coli K-12 when this organism was grown on these two media. An exception to this lack of influence of medium on the x-ray pattern characterizing a given test organism was encountered when a medium very high in salt concentration, such as Staphylococcus Medium No.110, was compared with a medium low in salt, such as Bhitsye, for the cultivation of S. aureus 3A5. The concentration of sodium chloride was considerably greater within cells grown on Staphylococcus Medium No.110 than on Bhitsye medium (ca. 1% NaCl), as shown by the x-ray diffractograms.

Effect of Method of Sample Preparation on Diffraction Patterns

1. Effect of cell extraction procedures:

   A. Autoclaving cell suspensions in deionized water, and subjecting cells to autolysis at 65°C, produced extracts that were not suitable in that crystallization of solutes was poor, and the resulting x-ray patterns were weak.

   B. Trituration of cells with crystalline urea, EDTA, or solvents was unsatisfactory because the added solvating agents often gave strong diffractive patterns that masked the weaker patterns produced by the crystalline materials in the cell extractives.

   C. Grinding dried cells with powdered Pyrex glass, leaching cells with cold deionized water, and alternate freezing and thawing, were not sufficiently rapid or efficient methods for removing solutes or for extracting solutes, without contaminating with excessive amounts of protein.

   D. Treatment of cell suspensions in the 10 KC Raytheon sonic oscillator was the best method found for production of suitable cell extracts for x-ray diffraction study.

      a. Buffering the cell suspension and making the salt concentration isotonic greatly improved the rate of cell shear, but was objectionable because of the masking effect of the diffraction patterns given by Tris buffer, or added mineral salts.

2. Effect of electrodialysis on diffraction patterns of cell extracts:

   When water soluble cell extracts were electrodialyzed against cold deionized water for several hours in the Raymond Electroconvection apparatus*, the fraction collecting in the cathodic upper chamber of the dialysis sac gave x-ray diffraction patterns that were not only much richer in strong diffraction peaks, but were markedly different from the patterns given by the untreated cell extract.

3. Effect of x-ray diffractometer settings on diffraction patterns:

   Relatively thick samples are required to effectively detect the weaker diffracting planes in crystalline cell extracts. The use of a vacuum radiation path, as suggested by Hoskins (1960) for x-ray spectrographic work, would possibly permit effective diffractometric examination of thinner preparations of cell extracts. Reduction in the required sample size would greatly facilitate this type of research, in which many biologically interesting materials are unavailable in the presently required gram-size samples.

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* E-C Apparatus Co., 23 Haven Ave., New York 32, N.Y.

Partial Identification of Diffraction Peaks Given by Cell Extracts

1. It was noted that some of the major diffraction peaks were nearly identical on diffractograms obtained with a wide variety of cell extracts; however, in addition to these common peaks, each material showed distinctive peaks.

2. Ashing the samples didnot destroy these common diffractive peaks, and the diffractive material could be obtained from intact bacterial cells by leaching in cold deionized water.

3. Reference to the Hanawalt File (1959) of x-ray diffraction data revealed that halite (sodium chloride) and sylvite (potassium chloride) gave x-ray diffraction patterns bearing a close resemblance to certain segments of the diffraction patterns of most cell extracts. When diffractograms were made of halite and silvite and compared with the diffractograms of selected cell extracts, it was apparent that the halite-like and sylvite-like peaks in the cell extract diffractograms were shifted or modified in shape, and in some cases missing entirely. These features may serve as distinguishing characteristics of specific cell extracts.

4. Within the 18-31° Bragg angle region, monochromatic copper radiation gave rise to a strong fluorescence with many cell extracts, presumably because of the large amount of iron or cobalt compounds present. These fluorescing compounds could be extracted from E. coli cells with EDTA solution, and could be concentrated in the cathodic fraction of electrodialyzed yeast extract. This behaviour would seem to indicate that the fluorescing compounds are positively charged molecules that are nonpermeating with respect to celloidin membranes; the ease of their removal from intact E. coli cells would seem to suggest that these iron or cobalt compounds are loosely bound to the surface structures of this organism. An iron target as the source of x-rays of a wavelength outside the critical absorption range of iron-cobalt compounds would prove very helpful in studying the structure of these x-ray fluorescent compounds.

DISCUSSION

The mechanism whereby different microbial forms can grow in the same chemical environment and yet produce markedly different patterns of solutes in their respective cytoplasms has puzzled cytochemists for many years. The most popular explanation of this ability is based on a selectively permeable membrane hypothesis. Cohen and Monod (1957) have proposed the term 'permease' to describe stereospecific and functionally specialized enzymes which control entry of metabolites into living cells. Rothstein (1959), in his excellent review, refers to the work of Conway and Dugan (1958) who postulate on the basis of azide and cyanide sensitivity that permeases are iron-containing enzymes which

ferry K^+ ion into the cell during active transport by reversible reduction-oxidation of the iron. Considering our results with iron or cobalt compounds in cell extracts, the interesting possibility presents itselt that an iron or cobalt compound, or compounds, may function as the coenzyme for cell permease, making this type of iron compound distinct functionally from the familiar iron-porphyrin enzymes of the cell.

Permease systems are very efficient, concentrating amino acids against a concentration gradient of 1 to 500 (Cohen and Rickenberg, 1956, 1958), and accumulating lactose in protoplasts to 22% of their dry weight (Sistrom, 1957). Permease behaviour toward inorganic ions is similar, as Rothstein (1959) reported that cells "can, within certain limits, maintain an internal electrolyte composition considerably different from that of the environment," and he stated further that "under conditions of maximal metabolism, certain ions are accumulated."

Mitchell and Moyle (1956) cite their own and many other workers' results as indicating that the internal concentration of solutes in halophilic bacteria may reach 30 atmospheres osmotic pressure, or approximately 1 M MaCl equivalent. Even the nonhalophilic bacteria may reach 13 atmospheres internal concentration of electrolytes. Flannery (1956) reports that for the halophilic organisms the sodium ion, and to a lesser extent, the chloride ion, are essential to the well-being of obligate halophiles, but Ingram (1957) found that halophiles range from fixed to facultative requirements for various ions.

Most cells are highly retentive of the electrolytes and other solutes which contribute to their high osmotic pressure, as indicated by the work with protoplasts (Weibul, 1955), but some cells have been shown to be 'leaky' losing cellular phosphate and potassium ions when washing with distilled water is carried out.

Our results with x-ray diffraction methods confirmed the reports that solutes are highly concentrated inside microbial cells, as shown by other analytical methods, but added the information that Na^+, K^+, and Cl^- are apparently present as such in cytoplasm since they are able to crystallize out as halite and sylvite. Furthermore, we were able to show that S. albus and S. aureus both leak NaCl and KCl when suspended in cold deionized water for as little as 48 hours, while under the same conditions Saccharomyces cerevisiae cells retained both these salts, as indicated by x-ray diffractograms of the dried wash solutions. Micrococcus flavus, however, was found to be able to retain substantially all its KCl, but to lose NaCl readily. These findings support the results obtained by other methods of analysis by Mitchell and Moyle (1956), Weibull (1955), and numerous other workers cited in these two references.

This x-ray diffractometric 'fingerprinting' of cell extracts has great potential as a cytochemical tool, as well as a taxonomic application. However, the effectiveness of this technique is seriously impaired by lack of a source file of x-ray diffraction data for components of cell extracts. Most of the peaks obtained on a diffractogram of cell extract can not be identified as to the crystal source, with our present limited information.

Removal of the halide salts which have such intense diffraction patterns, and concentrating the smaller highly charged molecules in the upper chamber of an electrodialysis apparatus, is a procedure which

improves the characteristics of cell extracts in diffractometric analysis. These electrodialyzed extracts give many more strong diffraction peaks than the untreated extracts, and are relatively free of masking effects by the salt patterns present in the original cell extracts.

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COMMERCIAL LAUNDERING OF WOVEN COTTON, LINEN, RAYON, AND CELLULOSE SECONDARY ACETATE[1]

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ABSTRACT. Standard constructions of apparel cloths, napery, sheeting, and toweling are analyzed for change occurring during 200 commercial launderings with soap and water. Dependence of change in acetyl, dimensions, strength, weight, whiteness, and residual ash of cloth on number of laundering is summarized as initial rate of change according to the pre-exponential parameter of the Tammann-Elovich equation for rate of chemisorption when time is replaced with number of laundering.

These summaries of change not only set apart excellent, superior, acceptable, and scarcely surviving cloths but also place similar cloths in order of stability against the composite process of laundering. Initial rate of change in strength-to-weight ratio of cloth provides approximate appraisal of durability.

Cotton cloths are compared with linen cloths over the course of the laundering according to relation of whiteness to residual ash, dimensional change to initial cover factor for yarn, weight to initial cover factor for cloth, and strength to weight.

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This account of change brought about during commercial laundering without soiling compares cotton broadcloth, muslin, and organdie both in loomed and in finished condition; other cloths woven plain and several satins different in fiber, finish, or weight; two flannels; and birdseye, fisheye, honeycomb, huckaback, sand crepe, and terry towelings. How these native or modified celluloses fared when laundered with soap and water is told as change in acetyl, dimensions, strength, weight, whiteness, and residual ash of cloth. Description of cloths before laundering is collected in Figures 1, 2, and 3 and Tables I, II, columns 1 and 4 of Table V, column 1 of Table VI, and columns 1 through 4 of Table VIII.

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[1] Journal Paper No. J-3704 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 552.

[2] Department of Chemistry, Oklahoma State University, Stillwater, Okla.

[3] Deceased.

Conditions of Laundering

Continuous yardage of cloth bought at retail was cut along filling yarns to a set of seven swatches; one swatch was saved for analysis and the other six, each stitched at cut edges and numbered with cotton thread, were bagged in cotton net for wet processing of 1, 25, 90, 125, 150, and 200 launderings in a ten-pocket mechanical washer. Table III lists operations of Cycle A for laundering all but the flannels and five apparel cloths. Temperature of wet processing was held below 50°C, for both flannels that were neither bleached nor blued; the ice crepe (27), organdies, rayon muslin, and taffeta were laundered under conditions set out as Cycle B (Table IV).

Methods of Analysis

Conditioning of textiles. Equilibration of samples with the more humid standard atmosphere was begun a week before physical testing according to ASTM Designation: D 39-59 (1).

Structure of yarn. Burning behavior and morphologic structure, along with solubility in acetone and basic deacylation of ester, confirmed commercial designations of fibers as bast, cellulose secondary acetate, cotton, and rayon. For lack of a way to differentiate among refined basts and failure in attempts to sort these basts according to direction of drying twist (20), flax stalks are assumed, rather than known, to have been the source of basts brought as linen.

Length of staple was approximated as mean length of ten fibers teased apart midway between ends of much longer yarns and measured on black velvet.

Linear density of yarn was defined by separate weighing to the nearer milligram of four bunches of yarn, each a total of 10 meters or more (14). Number of filaments listed for cross-sectional area of acetate or rayon yarn is an average of five counts under magnification.

For tenfold analysis of its spiral structure, taut yarn ten inches long at start of most measurements was untwisted by turning rotating jaws of an Improved Twist Tester from the United States Testing Company until a pick run between paralleled strands or fibers detected no twist.

Pattern of cloth. The pattern of interlacement was plotted with black square for warp yarn and blank square for filling yarn at front face. The smaller angle between filling yarn at front face of the Canton flannel and the Z-diagonal of its swansdown twill, as well as that between selvage and slant of Schreinersheen lines calendered across the front face of each cotton damask, was measured with a protractor.

Warp and filling yarns in an inch of cloth were counted at five areas no nearer selvage than one-tenth width of cloth and without warp or filling yarn in common. Schreinersheen lines on an inch of cotton damask were counted in five such areas.

To find the contribution of warp and filling to weight of cloth, four 3-inch or wider squares, no two including the same yarn and none nearer selvage than one-tenth width of cloth, were trimmed off along outer yarns before squares and their systems of yarn were weighed severally to the nearer milligram (13). For finding fraction of components in composite

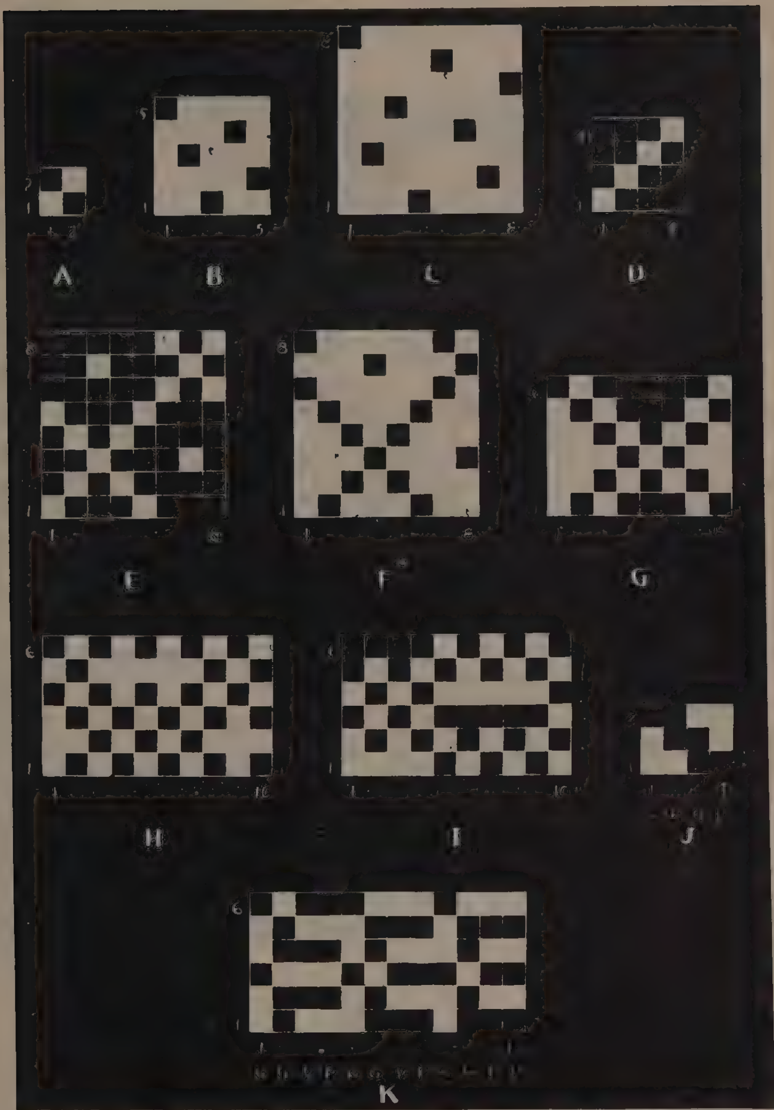

Figure 1. A, plain weave; B, 4/1 satin weave; C, 7/1 satin weave; D, 1/3 twill weave; E, F, G, diamond twill weaves; H, I, fancy rib weaves; J, K, pile weaves in which G is the ground yarn and P the looped pile.

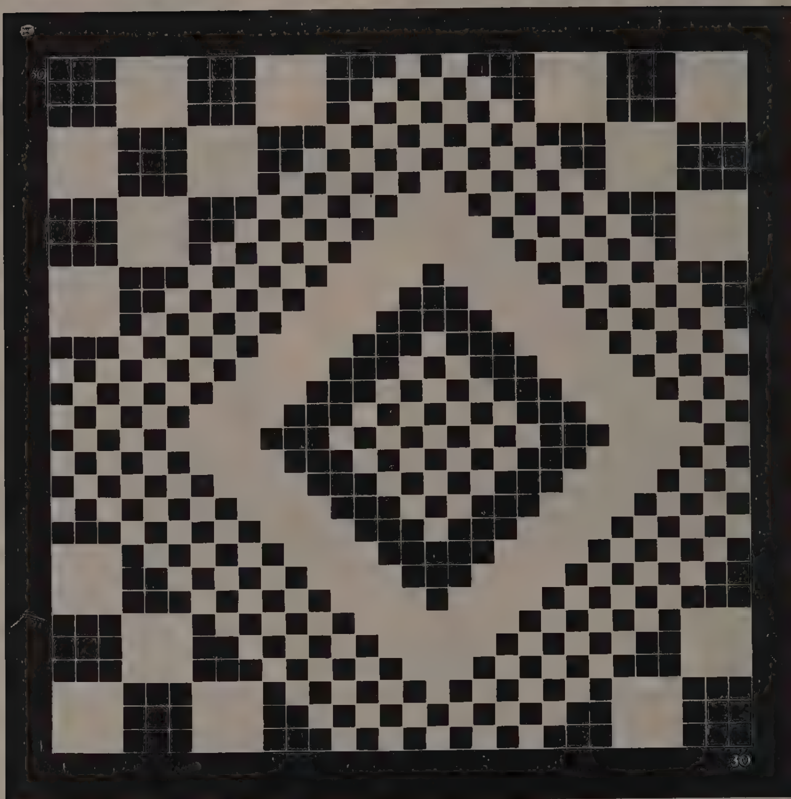

Figure 2. Diamond twill weave.

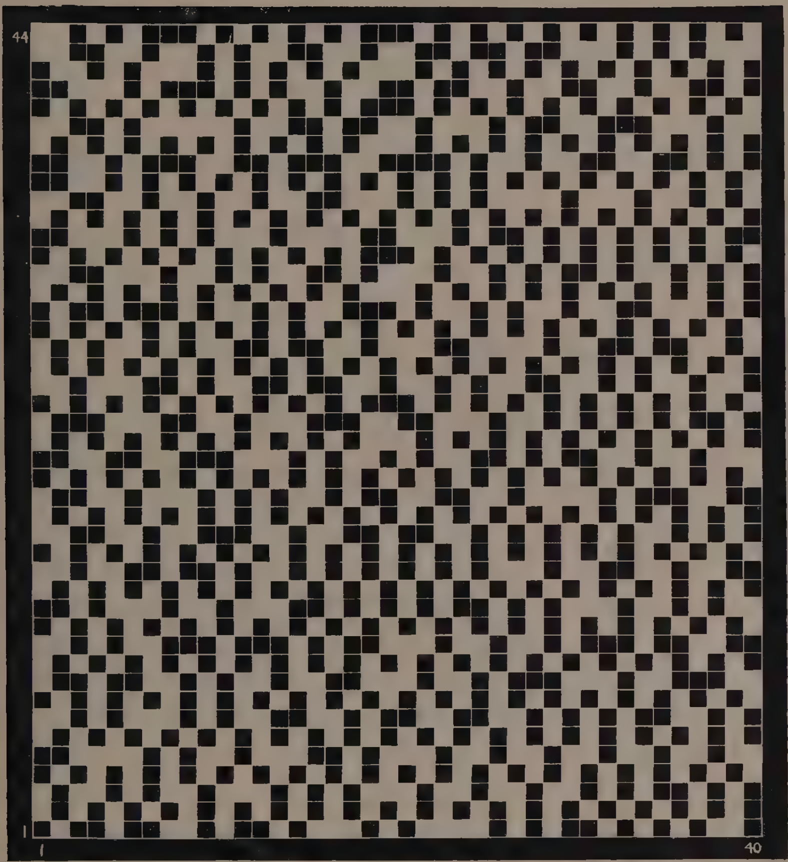

Figure 3. Sand crepe weave.

Table I. Structure of Cloth

Cloth number	Cloth	Weave	Cover factor: Warp (fraction of planar projection of cloth)	Filling	Cloth	Distribution of yarn in cloth: warp yarns/inch	filling yarns/inch	Warp gram/100 g. of cloth
1	Broadcloth, loomed	plain (Fig.1A)	0.780	0.376	0.863	125	69	71.6
2	Broadcloth, finished	plain	0.756	0.356	0.843	130	64	69.2
3	Ice crepe	plain	0.583	0.412	0.755	66	57	63.0
4	Muslin, rayon	plain	0.490	0.385	0.686	94	73	56.6
5	Organdie, loomed	plain	0.390	0.297	0.571	76	73	59.8
6	Organdie, finished	plain	0.415	0.303	0.592	89	81	61.4
7	Taffeta	plain	0.792	0.377	0.870	183	61	60.5
8	Bisso, linen	plain	0.366	0.351	0.588	59	58	50.5
9	Crash, linen	plain	0.499	0.551	0.775	42	47	48.1
10	Satin, linen	4/1 satin (Fig.1B)	0.670	0.547	0.850	80	65	55.5
11	Satin damask, linen	7/1 satin (Fig.1C)	0.708	0.804	0.943	81	100	46.1
12	Satin damask, mercerized cotton[a]	4/1 satin	0.506	0.576	0.791	64	55	41.8
13	Satin damask, mercerized cotton[b]	4/1 satin	0.617	0.665	0.872	79	77	44.1
14	Satin damask, rayon	4/1 satin	0.610	0.568	0.832	70	62	50.5
15	Sheeting, nonbleached cotton muslin	plain	0.556	0.572	0.810	68	72	51.1
16	Sheeting, bleached cotton muslin	plain	0.577	0.540	0.805	74	68	49.8
17	Sheeting, linen cambric	plain	0.582	0.554	0.814	77	77	53.2
18	Sheeting, Canton flannel	1/3 twill, 63° (Fig.1D)	0.592	0.503	0.797	88	41	43.4
19	Sheeting, cotton flannel	plain	0.285	0.453	0.609	33	30	30.0
20	Sheeting, percale	plain	0.627	0.584	0.845	104	103	51.9
21	Toweling, cotton birdseye	diamond twill (Fig.1E)	0.420	0.489	0.704	66	51	35.6
22	Toweling, linen birdseye	diamond twill (Fig.1F)	0.682	0.584	0.868	98	90	56.0
23	Toweling, linen fisheye	diamond twill (Fig.2)	0.740	0.488	0.867	82	67	65.7
24	Toweling, cotton honeycomb	diamond twill (Fig.1G)	0.524	0.376	0.702	31	22	55.0
25	Toweling, cotton huckaback	Devon huck (Fig.1H)	0.575	0.845	0.934	50	56	49.2
26	Toweling, linen huckaback	fancyrib (Fig. 1 I)	0.705	0.403	0.824	82	47	61.5
27	Toweling, linen sand crepe	sand crepe (Fig. 3)	0.990	0.654	0.997	79	55	59.4
28	Toweling, cotton terry	terry (Fig. 1 J)	0.352	0.397		30	33	23.2
	cotton pile yarn					28		60.2
29	Toweling, cotton and linen terry	terry (Fig.1K)	0.336	0.362		28	43	10.4
	linen pile yarn					27		81.0

[a] Calendered across front face with 225 parallel lines/inch at 75° to selvage
[b] Calendered across front face with 225 parallel lines/inch at 60° to selvage

Table II. Structure of yarn.

Cloth No.	Length of Staple: Warp yarn	inch	Length of Staple: Filling yarn	inch	Twist in Yarn: Warp yarn: Single direction, tpi	Warp yarn: Plying direction, tpi	Filling yarn: Single direction, tpi	Filling yarn: Plying direction, tpi	Linear density of yarn: Warp yarn tex	Filling yarn tex
1	Cotton	1.3	Cotton	1.3	Z 11.0	S 10.5	S 9.4		16.2	12.6
2	Mercerized cotton	1.3	Mercerized cotton	1.3	Z 9.5	S 10.5	S 11.0		14.2	12.9
3	Sheath of dull cellulose secondary acetate 54.2% and core of bright F60 rayon	5.0 / continuous	Sheath of dull cellulose secondary acetate 52.2% and core of bright F30 rayon	5.0 / continuous	S 54.2 / Z 42.2	S 13.8[a] / Z 12.0[a]	Z 40.1	Z 15.0	32.9	22.2
4	F60 bright rayon	continuous	F50 bright rayon	continuous	0.0	0.0	0.0		11.3	11.6
5	Cotton	1.3	Cotton	1.3	Z 21.2	Z 22.6	Z 22.6		10.8	6.99
6	Cotton	---	Cotton	---	Z ---	Z --	Z --		9.22	5.96
7	F20 bright cellulose secondary acetate	continuous	F46 bright cellulose secondary acetate	continuous	0.0	0.0	0.0		7.87	16.0
8	Linen	2.4	Linen	2.5	Z 10.6		Z 11.0		16.1	15.5
9	Linen	2.1	Linen	2.1	Z 7.0		Z 7.1		58.4	58.4
10	Linen	1.8	Linen	1.8	Z 12.8		Z 13.8		29.4	30.1
11	Linen	2.0	Linen	2.0	Z 10.6		Z 10.6		32.3	26.8
12	Mercerized cotton	0.9	Mercerized cotton	0.8	Z 18.0		Z 9.8		26.6	45.6
13	Mercerized cotton	0.8	Mercerized cotton	0.9	Z 18.4		Z 14.6		25.6	30.3
14	Bright rayon	1.2	F45 bright rayon	continuous	Z 1.0	S 15.8	S 1.7		31.5	35.6
15	Cotton	1.2	Cotton	1.2	Z 14.8		Z 10.4		28.0	26.5
16	Cotton	1.0	Cotton	0.9	Z 15.4		Z 12.4		25.4	26.2
17	Linen	1.6	Linen	1.8	Z 8.2		Z 8.3		24.7	21.9
18	Cotton	1.1	Cotton	0.8	Z 13.9		Z 10.2		19.1	62.9
19	Cotton	1.1	Cotton	0.9	Z 14.5		Z 5.2		31.3	92.7
20	Mercerized cotton	1.2	Mercerized cotton	1.2	Z 23.0		Z 25.5		15.4	13.6
21	Cotton	1.2	Cotton	1.2	Z 18.4		Z 18.8		17.1	38.2
22	Linen	2.0	Linen	2.0	Z 6.8		Z 6.9		20.4	17.9
23	Linen	2.2	Linen	1.9	Z 10.6		Z 10.6		34.0	22.4
24	Cotton	0.9	Cotton	0.9	Z 9.7	S 8.2	Z 10.1	S 7.3	119.0	121.
25	Cotton	1.0	Cotton	1.0	Z 13.4	S 10.0	Z 13.9	Z 0.9	54.7	97.7
26	Linen	2.2	Linen	2.2	Z 13.2		Z 12.8		31.2	31.4
27	Linen	1.9	Linen	2.0	Z 8.4		Z 8.3		66.5	59.8
28	Cotton ground	1.0	Cotton	1.0	Z 5.9	S 14.1	Z 5.9	S 14.1	59.4	42.6
	Cotton pile	1.0			Z 5.9	S 14.1			59.4	
29	Cotton ground	1.1	Cotton	1.1	Z 15.2	S 12.2	Z 16.8		61.2	29.4
	Linen pile	2.6			Z 6.6				116.	

[a] One S-twist-on-twist and one Z-twist-on-twist warp yarn in alternate succession across cloth.

yarns of the ice crepe, the bright rayon cores of each system were reweighed after stripping off their sheathing strands of dull ester.

Since distortion of yarn in plane of cloth compensates for most change in spacing at interlacement, cover factor for cloth is derived as difference between sum and product of cover factors for its warp and filling systems. Projection of aligned warp or filling on a parallel plane is estimated (3, 10) through Ashenhurst's reciprocal of $0.92/\sqrt{\text{yds of yarn/lb}}$.

Height of terry pile was measured as ratio between mean length of ten warp yarns forming rows of projecting loops and mean length of ten coplanar warp yarns separated from the same square cut of cloth. This ratio and its standard error before laundering turned out to be 2.76 ± 0.02 in the cotton and 4.44 ± 0.01 in the semilinen terry.

Acetyl of cellulose secondary acetate. Four 2-gram squares of cloth freed of outer yarns for a centimeter from each edge were dried in tared weighing bottles for 90-minute periods in an electric oven at 105 - 110°C until consecutive weighings of a square were the same within 0.4 mg. Each square was plasticized at 50-60°C with 40 ml of 75% ethanol in stoppered glass flask before admixture of equal volume of 0.25 N sodium hydroxide for fibrous deacylation during 15 minutes at 50-60°C and two days at temperature of laboratory. These hydrolyzates and four blank determinations were made slightly acidic to phenolphthalein 2 hours before neutralization with standard acid (6, 7).

Ash of cloth. A 5-gram sample of cloth without selvage was dried the same way and ignited until constant in tared ceramic crucible at dull red heat of an electric furnace. Any dark residue was wet with nitric acid, dried, and reignited to remove carbon. In no instance is a value for ash averaged over fewer than three determinations.

Breaking strength of cloth. Within the same week soon after the last laundering, raveled-strip strength of all cloth was tested tenfold on a constant-rate-of-traverse Scott Tester. Before serial testing, the set of parallel strips to be broken wet was soaked 2 hours in 2 liters of distilled water at ambient temperature.

Dimensional change. Dimensional change in cloth is computed from numbers of warp and filling yarns in an inch of cloth before laundering and corresponding counts in laundered cloth. Shrinkage of terry-pile yarn is reckoned from warpway shrinkage of cloth and heights of pile in cloth before and after laundering.

Photoelectric measurement of cloth. Off-white is described in terms of light absorbed when cloth was stacked in enough layers to avoid measurement of background. Referred to black and white ceramic surfaces, the electric equivalent was read in four areas from a Pfaltz and Bauer Reflectometer Universal Model M U (17).

Weight of cloth. Three samples the width of cloth and somewhat more than 4 inches warpway were cut along filling yarns and weighed separately to an accuracy of a milligram. Without being stretched, each weighed sample was spread flat for measurements of length along five warp yarns rather equidistant across the cloth; whole width of cloth between outermost yarns of selvage was measured along filling yarn at right angles to warp.

Computation of observations. One extreme in a set of observations rounded for significance (18) was rejected whenever ratio between differ-

ence of two smallest or two largest and range exceeded the Dixon criterion at 10% level of acceptance (5).

Review of Results

The criterion taken for regular change in acetyl, dimension, strength, weight, whiteness, or residual ash throughout the laundering is a variant of the Tammann-Elovich expression for rate of chemisorption (21, 8),

$$dq/dn = A \exp(-\alpha q)$$

in which q is change at

n cycle of laundering,
A is initial rate of change, and
α, reciprocal of A, is a parameter computed as product of $\log_e 10$ and inverse slope of least squares-adjusted graph for q against $\log_{10}(n + 1)$.

How well this empiric equation reproduces analytic estimate of change is shown as standard error of initial rate (columns 3 and 6, Table V; columns 9 and 10, Table VI; columns 1 through 4, Table VII; and column 5, Table VIII).

Partial deacylation of ester. The ice crepe, 21.21 ± 0.13% acetyl before laundering, lost more cellulose than acetyl as evident from positive value obtained for initial rate in acetyl, 0.09 ± 0.09% of laundered cloth/laundering. Precision in heterogeneous analysis of the taffeta, 38.52 ± 0.10% acetyl before laundering, is within that claimed in homogeneous analysis for acetyl (15). Cumulative loss in acetyl, starting at -0.09 ± 0.03% of laundered cloth/laundering, came to 1.92% of the taffeta quite as reported by Jeffries (12).

Change in residual ash of cloth. Standard error for residual ash from laundered cloth describes rather even distribution of ash-forming increment except in the cotton honeycomb and linen crash (column 1, Table V). Most rates for rise in ash are slow as if ccumulation of ash-forming substance had been kept minimum through effective colloidal condition of soap-water systems, avoidance of large ionic concentration and excessive oxidation, and achievement of desorption during acidic rinse (column 2, Table V). Retention of ash-forming substance in the cotton terry, linen crash, and rayon damask was not accompanied with tattletale gray (columns 1, 2, 4, and 5, Table V). Coefficient of correlation between change in absorption of light and change in ash during 200 launderings was only 0.231 over all cottons except the honeycomb and huckaback and 0.360 over all linens except the crash. Although sorption at their accessible surfaces may account for whatever dinginess was acquired by other cloths, some 36-fold increment of ash-forming substance in the honeycomb would seem to indicate accretion through rinse-resistant occlusion within its bulky cotton yarns.

Dimensional change. Main dimensional change often came when strain in yarns was redistributed as fibers swelled reversibly during first laundering (columns 5 and 7, Table VI). Total shrinkage of terry-pile

Table III. Cycle A

Operation	Level of liquid at 48-inch diameter of 84-inch cylinder	Hydrogen-ion concentration	Temperature	Time
	inch	pH unit[a]	°C	minute
1. Rinse in water[b]	4		16 to 21	2
2. Dilute suds[c]	4	11 to 12	38	5
3. Concentrated suds[c]	3	11 to 12	60	7
4. Concentrated suds	3	11 to 12	71 to 77	7
5. Bleach containing 2 qt. of Javelle water, 0.75 per cent as to available chlorine	3	11 to 12	71	7
6. Rinse in water	10		77	3
7. Rinse in water	10		77	3
8. Rinse in water	10		77	3
9. Rinse in water	10		49	3
10. Bluing rinse	10	5.0 to 5.5	27	3
11. Centrifugal extraction until residual moisture was half the weight of cloth				18
12. Ironing			164	

[a]Initial pH was brought between 5.0 and 5.5 with fluosilicate and between 11 and 12 with washing soda.

[b]Initial ratio between weight of water and weight of cloth was 25.

[c]Initial ratio between weight of soap and weight of cloth was 5.0×10^{-3} for dilute and 7.5×10^{-3} for concentrated suds.

Table IV. Cycle B

Operation	Level of liquid at 48-inch diameter of 84-inch cylinder	Hydrogen-ion concentration	Temperature	Time
	inch	pH unit[a]	°C	minute
1. Rinse in water[b]	4		16 to 21	2
2. Dilute suds[c]	3	11 to 12	38	5
3. Concentrated suds[c]	3	11 to 12	38	5
4. Concentrated suds	3	11 to 12	38	5
5. Rinse in water	8		38	2
6. Rinse in water	8		38	2
7. Rinse in water	8		38	2
8. Rinse in water	8		38	2
9. Rinse	5	5.0 to 5.5	21	3
10. Centrifugal extraction until residual moisture was half the weight of cloth				18
11. Ironing			164	

[a]Initial pH was brought between 5.0 and 5.5 with fluosilicate and between 11 and 12 with washing soda.

[b]Initial ratio between weight of water and weight of cloth was 25.

[c]Initial ratio between weight of soap and weight of cloth was 5.0×10^{-3} for dilute and 7.5×10^{-3} for concentrated suds.

Table V. Residual Ash and Absorption of Light by Cloth

Cloth	Residual ash of oven-dry cloth				Initial rate of change in residual ash		Absorption of light by cloth		Initial rate of change in absorption of light
Cycle of laundering:	0		200				0	200	
number	gram/kilogram of initial cloth		gram/kilogram of initial cloth		p.p.t./ laundering		per cent	per cent	per cent/ laundering
1. Broadcloth, loomed	10.25	± 0.15	1.54	± 0.00	-0.06	± 0.03	15.6	17.4	0.66
2. Broadcloth, finished	0.74	0.10	2.47	0.02	0.29	0.05	7.0	12.4	0.60
3. Ice crepe	12.37	0.06	9.48	0.05	-0.58	0.10	12.0	12.0	1.06
4. Muslin, rayon	21.15	0.10	18.35	0.12	0.31	0.04	11.6	10.0	-0.17
5. Organdie, loomed	8.72	0.05	2.67	0.01	0.36	0.13	12.4	11.0	0.17
6. Organdie, finished	1.75	0.05	1.18	0.03	0.14	0.01	19.6	18.0	-1.47
7. Taffeta	0.84	0.04	2.13	0.04	0.38	0.05	8.0	15.0	0.11
8. Bisso, linen	7.94	0.08	3.45	0.32	-0.40	0.02	16.6	15.0	-0.79
9. Crash, linen	4.42	0.00	21.28	1.32	1.87	0.54	17.0	13.0	-0.20
10. Satin, linen	0.95	0.12	1.66	0.06	0.14	0.06	4.4	5.0	0.64
11. Satin damask, linen	0.84	0.10	3.67	0.06	0.20	0.06	0.0	5.0	1.9
12. Satin damask, mercerized cotton	1.28	0.05	1.47	0.07	0.18	0.02	5.0	8.8	0.60
13. Satin damask, mercerized cotton	1.48	0.06	2.07	0.00	0.22	0.02	2.4	13.6	0.06
14. Satin damask, rayon	2.47	0.09	8.06	0.09	0.75	0.11	14.6	11.6	-0.89
15. Sheeting, nonbleached cotton muslin	8.74	0.70	2.28	0.09	-0.07	0.04	15.0	15.0	-0.32
16. Sheeting, bleached cotton muslin	0.09	0.00	2.25	0.05	0.23	0.02	4.8	13.2	1.5
17. Sheeting, linen cambric	2.53	0.07	1.46	0.07	-0.02	0.01	9.6	12.0	0.64
18. Sheeting, Canton flannel	0.66	0.10	2.41	0.05	0.30	0.02	5.2	14.8	2.2
19. Sheeting, cotton flannel	8.07	0.06	2.61	0.06	0.26	0.04	16.6	13.2	0.20
20. Sheeting, percale	0.62	0.05	2.02	0.03	0.23	0.01	6.4	14.0	0.15
21. Toweling, cotton birdseye	0.26	0.02	2.21	0.00	0.22	0.02	5.6	10.0	-0.25
22. Toweling, linen birdseye	2.00	0.00	2.86	0.02	0.13	0.06	8.6	10.0	0.87
23. Toweling, linen fisheye	1.22	0.14	3.25	0.30	0.19	0.03	4.6	8.6	0.72
24. Toweling, cotton honeycomb	1.70	0.58	61.31	1.79	9.78	2.18	9.2	16.0	0.93
25. Toweling, cotton huckaback	3.90	0.34	8.32	0.44	1.44	0.15	9.4	14.2	1.1
26. Toweling, linen huckaback	1.70	0.14	1.57	0.03	-0.03	0.01	6.0	11.4	0.41
27. Toweling, linen sand crepe	2.41	0.05	2.98	0.04	-0.14	0.03	15.0	16.0	-0.42
28. Toweling, cotton terry	0.93	0.04	1.57	0.03	0.22	0.02	34.0	14.0	-1.5
29. Toweling, cotton and linen terry	6.19	0.11	2.48	0.04	0.23	0.05	75.0	12.0	-0.49

Table VI. Change in Weight and Dimensions of Cloth

Cloth	Weight of cloth		Initial rate of loss in weight		Dimensional change in cloth[a]				Initial rate of shrinkage	
					Warpway		Fillingway		Warpway	Fillingway
Cycle of laundering:	0	200			1	200	1	200		
number	ounce/ square yard	per cent of initial	per cent of initial cloth/ laundering	per cent of laundered cloth/ laundering	per cent	per cent	per cent	per cent	per cent/ laundering	per cent/ laundering
1	3.81	84.5	0.67	0.12	9.7	9.3	1.6	4.4	0.17	0.50
2	3.28	98.1	1.25	0.58	- 0.9	2.1	1.1	2.2	0.6	0.09
3	4.76	105.	-1.44	-2.70	21.	22.	12.	14.	0.07	0.35
4	2.27	98.1	1.25	0.67	- 0.5	3.2	2.9	2.7	0.7	-0.08
5	1.68	85.9	0.06	-1.51	7.4	11.	2.6	5.2	0.73	0.67
6	1.60	95.6	0.73	0.31	1.9	3.6	7.1	9.2	0.15	0.29
7	2.88	105.	0.02	-2.04	2.5	11.	1.7	1.1	1.9	-0.10
8	2.22	66.1	5.50	5.50	13.	14.	1.7	8.1	-0.04	1.2
9	6.42	77.1	3.37	3.41	5.3	6.0	7.8	9.4	0.12	0.31
10	5.35	78.6	1.96	1.95	4.2	5.3	2.2	2.2	0.30	-0.21
11	6.38	74.1	4.18	2.34	6.9	10.3	-2.2	1.	0.2	0.95
12	4.85	94.0	0.94	1.32	2.8	3.5	2.8	4.2	-0.24	-0.09
13	5.41	89.6	1.84	1.50	4.2	4.9	2.2	3.9	0.24	0.22
14	5.21	96.0	0.11	0.04	4.3	5.2	2.2	0.6	0.19	-0.12
15	5.10	86.5	1.52	0.34	7.2	9.5	5.8	9.3	0.59	0.69
16	4.61	89.1	1.65	2.49	5.8	4.2	-0.8	-5.7	-0.35	-0.69
17	4.51	90.8	1.95	1.65	0.3	-1.8	0.5	1.8	0.4	0.00
18	4.78	100.	0.54	-0.87	1.9	2.3	-2.7	0.9	0.60	0.68
19	4.50	90.0	2.10	-1.34	- 3.3	5.6	-1.2	4.6	2.1	1.4
20	3.79	95.5	1.29	0.42	4.1	5.7	1.1	2.1	0.26	0.73
21	4.31	91.8	3.58	3.16	3.8	7.6	-3.0	-2.7	0.91	0.19
22	4.54	85.5	2.69	1.60	5.1	5.5	2.6	7.4	0.26	1.0
23	5.13	83.8	2.93	2.53	0.1	2.6	3.1	1.4	0.4	0.32
24	8.23	93.9	1.60	-2.54	4.3	19.	0.6	5.5	2.7	0.57
25	6.92	93.8	1.55	-2.34	2.1	10.	3.1	7.0	2.4	1.0
26	4.88	83.3	3.19	2.37	4.9	4.5	0.0	5.3	0.23	0.85
27	11.37	81.8	2.21	1.95	5.2	4.2	-2.3	-0.3	-0.21	0.56
28	9.25	87.3	0.71	0.60	9.7	8.7	4.3	6.2	0.18	0.38
Cotton pile					7.2	17.6			2.1	
29	19.17	79.9	2.29	1.43	10.	9.2	2.5	9.5	-0.39	1.6
Linen pile					18.4	25.1			1.6	

[a]Negative sign shows relaxation

Table VII. Change in Breaking Strength of Yarn in Cloth.

number	Cloth	Initial rate of loss in strength: Warp yarn, Conditioned per cent/ laundering		Warp yarn, Wet per cent/ laundering		Filling yarn, Conditioned per cent/ laundering		Filling yarn, Wet per cent/ laundering		Remanent strength at 200 launderings: Warp yarn, Conditioned per cent of initial	Warp yarn, Wet per cent of initial	Filling yarn, Conditioned per cent of initial	Filling yarn, Wet per cent of initial
1	Broadcloth, loomed	2.98 ±	0.32	4.60 ±	0.93	2.03 ±	1.29	2.80 ±	0.67	69.3	68.0	89.5	72.5
2	Broadcloth, finished	2.23	0.58	4.08	0.65	13.5	0.6	9.75	0.88	79.8	66.9	90.0	65.9
3	Ice crepe	4.09	0.20	5.75	1.08	8.73	0.80	6.05	0.96	41.6	51.1	46.2	42.6
4	Muslin, rayon	9.51	0.58	13.6	0.4	10.7	0.9	12.2	0.9	57.8	58.6	69.5	60.3
5	Organdie, loomed	1.70	0.24	5.21	0.33	3.82	0.61	4.55	0.38	63.3	71.4	78.4	68.4
6	Organdie, finished	3.81	0.67	2.57	0.28	2.76	0.44	-1.85	0.44	90.2	97.7	85.4	83.6
7	Taffeta	12.3	1.8	15.1	2.0	18.4	1.9	17.1	1.5	48.8	38.3	12.1	18.4
8	Bisso, linen	3.38	0.66	11.4	1.5	9.54	0.79	17.9	1.5	16.8[a]	3.8[a]	6.0[a]	4.1[a]
9	Crash, linen	7.14	0.88	12.4	1.4	7.53	0.96	10.9	0.5	43.1	35.1	49.7	28.7
10	Satin, linen	6.21	0.32	9.04	0.64	13.0	0.4	8.24	0.59	75.3	45.1	56.7	37.6
11	Satin damask, linen	8.89	0.37	12.7	0.9	12.7	1.4	14.1	1.8	36.4	21.8	20.8	17.0
12	Satin damask, mercerized cotton	2.63	0.45	3.13	0.33	2.99	0.35	6.00	0.54	67.0	80.6	72.0	68.4
13	Satin damask, mercerized cotton	5.51	0.66	4.87	0.27	2.43	0.59	7.64	0.93	70.7	68.8	86.3	74.8
14	Satin damask, rayon	20.2	0.9	17.8	1.3	3.46	0.88	9.81	1.41	3.5	0.8	85.7	45.4
15	Sheeting, nonbleached cotton muslin	4.44	0.62	5.98	0.52	1.74	0.29	4.63	0.57	81.7	73.0	81.4	64.4
16	Sheeting, bleached cotton muslin	3.93	0.55	5.55	0.70	4.97	0.75	9.18	0.94	63.2	48.1	54.1	37.0
17	Sheeting, linen cambric	5.33	0.61	9.08	0.41	6.59	0.38	5.39	1.03	63.2	55.6	70.5	50.6
18	Sheeting, Canton flannel	7.91	0.19	6.04	0.40	-4.82	0.79	4.43	1.03	67.2	63.6	141.	100.
19	Sheeting, cotton flannel	2.83	0.34	1.44	0.19	-27.7	3.8	7.29	1.41	74.5	61.6	283.	127.
20	Sheeting, percale	2.64	0.49	4.35	0.26	3.61	0.51	7.74	1.44	84.9	73.3	91.9	84.0
21	Toweling, cotton birdseye	4.34	0.23	9.59	0.16	5.19	0.39	7.02	0.32	74.7	48.0	76.0	59.5
22	Toweling, linen birdseye	8.06	0.63	14.3	1.0	8.72	0.70	1.54	0.77	48.9	37.0	42.9	18.5
23	Toweling, linen fisheye	10.5	0.7	7.54	1.53	11.4	1.7	24.1	1.9	36.2	21.7	25.3	14.7
24	Toweling, cotton honeycomb	9.37	0.27	7.14	0.92	12.8	0.6	15.6	1.0	25.6	26.7	41.3	26.0
25	Toweling, cotton huckaback	5.13	0.45	9.08	0.63	5.45	0.28	6.31	0.54	61.5	54.3	69.8	63.0
26	Toweling, linen huckaback	4.57	0.45	8.77	0.82	8.96	1.12	13.7	1.0	81.1	49.8	47.2	41.9
27	Toweling, linen sand crepe	8.52	1.06	8.88	0.91	5.22	1.35	6.59	0.83	62.5	36.3	90.3	56.2
28	Toweling, cotton terry	5.44	0.66	3.47	0.18	1.31	0.34	4.20	0.95	78.2	76.2	79.5	70.8
29	Toweling, cotton and linen terry	-0.38	0.51	4.86	0.17	8.14	1.20	0.73	0.54	87.4	71.8	96.5	81.3

[a] At 125 launderings

yarns, 17.6% by double-strand cotton but 25.1% by single-strand linen of slack twist, and their fast rates of shrinkage accentuate dependence of dimensional instability on mobility of fiber and yarn in cloth (columns 6 and 9, Table VI). Crowding or overriding of yarns, forced when their crowns flattened during ironing, was favored by three structural relations: same direction for twist in warp and filling (26), farther floating of yarn, and divergent directions for diagonal of twill and twist in yarn (4, 11). The first arrangement allowed more compaction of warp with filling at crossover where direction of twist was the same at crossing surfaces; the latter arrangements gave yarn more freedom for movement until fixed by felting. Among cloths woven plain, the more compact shrank less as shown by negative slope of cumulative change warpway against cover factor for filling, -0.22 ± 3.88 for the cottons but -59 ± 19 for the linens, or cumulative change fillingway against cover factor for warp, -8.1 ± 3.7 for the cottons but -26 ± 16 for the linens. The corresponding correlations were 0.01 warpway and 0.43 fillingway for the linens.

Change in weight of cloth. No differential change in weight among systems of yarn was detected when laundered cloth and its warp and filling were weighed separately; this finding is in keeping with results from a statistical treatment of home laundering (19). Cumulative loss in weight by an area of cloth came to 16% of the nonbleached and 2-13% of the bleached cottons, 9-34% of the linens, 2-4% of the rayons, and 20% of the semilinen terry (column 2, Table VI). Although most loss in the first laundering was lubricant and sizing, mechanical action during repetitive alternation of swelling and oxidative bleaching with ironing caused continuing attrition of hydrophilic fibers. Compactness of plain-weave cloth was far more effective in conserving weight of linen than of cotton as shown by negative slope of cumulative loss in weight against cover factor for cloth, -0.933 ± 0.244 for linens contrasted with -0.025 ± 0.051 for cottons. Linting as bast tissues split or splintered and their intercellular substances washed away can account amply for large loss by linen as cloth loosened and thinned but gains by the ice crepe and taffeta are ascribed to underestimate of count as result of occasional overfolding and fusing of one thermoplastic filament atop another. Slight gain at some cycles by nine other cloths may have been due to irregular shrinkage, wrong estimate of dimensional change from counts in successive swatches rather than at the same site in a single swatch, or retention of solute. Regain of moisture during re-equilibration of laundered cloth retaining hygroscopic solute was more but different for the different fibers (Table IX). Correlation between rate of loss in weight and cover factor for plain-weave cloth was better for linens than for cottons, 0.970 as against 0.252 (column 3, Table VI).

Change in strength of cloth. None of the six swatches of any cloth appeared distorted, scarred, or scorched when the series of launderings ended although various cloths showed the higher wet strength of more crystalline cellulose, the lower wet strength of many modified celluloses, and whatever gain in strength had accompanied compaction and been favored through felting when friction fluffed protruding fibers, split filaments, and floating yarns. Poor precision, conspicuous among brittle basts far from the same in composition, dimensions, and structure, is

Table VIII. Breaking Strength of Initial Cloth

Cloth	Warpway strength				Fillingway strength				Initial rate of decrease in wet strength/unit weight of laundered cloth	
	Conditioned		Wet		Conditioned		Wet			
	pound/inch		pound/inch		pound/inch		pound/inch		per cent/laundering	
1	72.4 ±	1.6[a]	69.4 ±	0.3	29.8 ±	1.3	41.7 ±	0.3	3.75 ±	0.83
2	65.1	1.1	70.1	1.6	26.1	1.0	38.0	0.6	5.22	0.67
3	32.2	0.2	14.2	0.5	19.8	0.5	9.9	0.3	5.60	0.62
4	33.5	1.0	11.8	0.4	25.5	0.8	9.0	0.3	11.8	0.5
5	29.2	1.0	30.0	0.4	15.4	0.8	19.2	0.2	5.99	0.28
6	34.2	0.8	32.9	1.3	16.6	0.6	15.8	0.3	0.62	0.26
7	29.8	0.6	17.8	0.4	22.0	0.0	11.6	0.3	15.8	1.40
8	44.9	0.5	46.5	1.9	40.6	0.6	42.2	0.7	12.3[b]	1.1
9	79.5	3.5	88.7	3.6	102.0	2.3	141.9	1.4	9.46	1.11
10	80.4	2.2	122.7	4.2	57.5	4.7	63.0	1.4	8.16	0.50
11	111.9	1.5	99.5	4.0	116.3	4.0	94.0	4.0	12.4	1.2
12	51.2	0.7	44.7	0.6	55.8	1.5	48.4	0.8	3.53	0.20
13	54.2	1.5	59.6	0.6	61.7	0.7	57.7	1.3	4.80	0.41
14	120.8	2.1	105.7	4.7	41.8	0.3	11.7	0.2	16.7	1.2
15	57.9	1.0	64.8	0.5	64.9	1.4	80.1	1.5	4.44	0.43
16	47.3	0.6	44.3	0.8	47.8	1.8	45.8	1.0	6.75	0.45
17	73.7	1.0	85.8	2.4	67.2	1.6	74.9	3.1	7.83	0.46
18	59.3	0.8	54.4	0.8	18.4	1.3	22.9	0.7	5.37	0.39
19	26.5	1.3	32.8	1.0	7.0	0.3	18.0	0.6	-3.47	0.92
20	56.4	1.1	57.3	2.1	51.5	0.8	44.6	2.3	4.96	0.63
21	29.6	0.9	38.3	0.3	51.7	1.3	57.2	0.5	5.50	0.22
22	92.6	3.6	101.1	4.4	64.5	1.5	71.2	1.7	13.4	0.7
23	101.1	2.0	114.7	2.0	45.1	1.8	44.3	0.8	14.4	1.0
24	69.1	1.0	85.4	2.1	30.4	0.7	45.7	0.8	10.4	0.6
25	80.1	1.5	84.6	1.4	71.9	1.5	76.1	1.8	7.67	0.55
26	74.4	0.9	90.9	1.9	64.9	1.5	68.4	4.5	9.13	0.85
27	152.7	3.2	229.1	2.8	81.1	2.3	156.4	4.0	6.77	0.76
28	33.7	0.8	44.2	0.7	32.5	0.5	39.7	0.9	3.22	0.33
29	33.5	0.3	52.9	0.7	33.6	1.3	41.0	0.6	0.87	0.73

[a] Standard error of mean
[b] Computed for the first 125 launderings

Table IX. Sorption of Water Vapor at 25°C as Function of Relative Pressure.

Reference	Fiber	Relative pressure p/p_o		Slope of isotherm gram of water/ gram of fiber against p/p_o	
22	Cellulose secondary acetate	0.05	to 0.65	0.0882	± 0.0008
24	Cotton	0.05	0.65	0.0874	0.0003
23	Cotton	0.05	0.621	0.0943	0.0010
22	Cuprammonium rayon, desoaped Bemberg	0.05	0.65	0.162	0.001
16	Flax, fine hackled[a]	0.443	0.643	0.0895	0.0019
25	Mercerized cotton	0.006	0.615	0.156	0.003
22	Viscose rayon (Vistra staple)	0.05	0.65	0.160	0.001

[a] At 21°C.

not unexpected since breaking strength of assemblage is a complex variate (Table VIII).

The broad range for change in strength of yarn in cloth brings out interplay among composition, contour, finish, linear density, spirality, width, interlacement, and spacing of yarns for satisfactory survival (columns 5 through 8, Table VII). A mechanical criterion for failure of cloth in use, loss of half its breaking strength (9), underrates strong cloths even more severely when interpreted in terms of yarn in cloth.

Larger standard error for rate of change in strength of yarn in wet cloth than in conditioned is ascribed to more sensitive detection of degradation by the former (columns 1 through 4, Table VIII). Correlation of rate for change in strength of yarn in wet cloth with rate for change in weight of unit area of cloth was 0.450 warpway and 1.00 fillingway for all cottons but 0.403 warpway and 0.390 fillingway for all linens (column 3, Table VI).

That felting was a cause for rise in fillingway strength of three cottons seems to be shown both by sequence of positive values obtained for rate of change in strength of yarn in conditioned cloth, 2.03 ± 1.29%/laundering of the loomed broadcloth, 4.82 ± 0.79%/laundering of the Canton flannel on which direction of twist crossed Z-diagonal of swansdown twill at napped face (4, 11, 2) and 27.7 ± 3.8%/laundering of the flannel napped at both faces, and by sequence of remanent strength conditioned or wet (columns 3, 7, and 8, Table VII).

Rate of change in ratio between strength of wet cloth, summed over warpway and fillingway means as pound/inch, and conditioned weight of laundered cloth as ounce/square yard serve to compare survival among these cloths used almost always as laundered without return to first

dimensions (Column 5, Table VIII). Higher wet than conditioned strength for unit weight continued among cottons all launderings of the flannel, honeycomb, terry, and loomed organdie and more than 150 launderings of the nonbleached muslin, 125 launderings of the huckaback and finished broadcloth, and 25 launderings of the birdseye, loomed broadcloth, and heavier damask that had been advertised as stabilized by durable finish. Higher wet than conditioned strength for unit weight of linens lasted all launderings of the sand crepe and beyond 150 launderings of the crash, 90 launderings of the bisso and cambric, and 25 launderings of the birdseye, fisheye, huckaback, and 4/1 satin that was not so susceptible to snagging as the 7/1 satin sometimes styled double damask after the farther floating of its yarns.

This analytic evaluation of destructive attack during laundering neither tells the tactile and visual appeal of the various textures nor discriminates between contribution to breakdown by hidden cause, whether catalytic contaminant or structural flaw perhaps ever in cloth, and that by chemical, mechanical and thermal action. But aside from some haphazard weakening coupled with erratic scatter among replicate tests, the patterns of change are rather regular and describe durability against laundering as strongly dependent on geometric structure of yarn and cloth.

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ESTIMATION OF CORN MATURITY IN IOWA[1]

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SUMMARY: Data collected by the Iowa Crop and Livestock Reporting Service and the U.S. Weather Bureau, cooperating with the Weather Division, Iowa Department of Agriculture were used to study corn maturity in Iowa. These data were the yearly state averages for corn silking, and the estimated percent of corn that matured without frost damage.

Using those years when the average June temperature was below 70°F, the following regression equation was computed.

$$\hat{Y} = -227.8 + 3.35X_1 + 0.40X_2 \qquad R = 0.78^{**} \qquad s_{y.12} = 8.1\%$$

where $\hat{Y}$ = predicted percent of corn matured
X_1 = state average June temperature, °F
X_2 = effective frost date (Oct. 5 = coded value of 219).

Data from a few years where only 90-95% of the corn matured, but the June temperature averaged above 70°F, were not included under this procedure. In 1928 and 1958 the results using this method were extremely poor. However, in these two years the discrepancy would have been noticed at the date of 75% silking.

Data from those years in which the corn fields were 75% silked July 30 or later, and the number of days from silking to the first freeze were used to compute a regression on corn matured. The relationship was

$$\hat{Y} = 212.4 - 1.14X_3 + .73X_4 \qquad R = 0.91^{**}, \qquad s_{y.34} = 5.1\%$$

where $\hat{Y}$ = predicted percent of corn matured
X_3 = average date of silking
X_4 = days from silking to average freeze date.

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Soft corn results when field corn is killed by freezing before reaching maturity. Freezing temperatures kill the immature plant and stop further development of the kernels. The two properties of soft corn of most interest from an economic standpoint are its reduced dry matter content and its high moisture percentage. The first property results in lower test weight and yield. The second creates a storage problem if corn is picked too early and/or if weather conditions following freezing are unfavorable for drying. It is essential that early cognizance be taken in a "soft corn year" of the situation, and consideration be given to any action which should be taken in handling this corn.

Certain investigations (Shaw and Thom (7), Hallauer (2), Iowa Department of Agriculture (4))have indicated that a relatively constant period is necessary after silking for the corn to be safe from frost. Data on the silking time of the corn crop would thus appear to be useful in considering this problem. Information on silking dates is available from the Iowa phenology data collected by the Weather Division, Iowa Department of Agriculture (4), and the Iowa Crop and Livestock Reporting Service. The particular data used in this study were answers to the question, "Date when 75% of main field was silked?". The yearly means of this date will hereafter be referred to as the silking date in this paper.

No precise data have been collected on the date when the corn has reached its maximum dry weight (mature). Use of moisture data does not appear too promising because of the variability in moisture content between varieties, and even within a variety between years (2, 7). The Iowa Crop and Livestock Reporting Service has collected data on crop progress, farm operation progress etc. for a number of years. On November 1st of each year, an estimate of the percent of corn matured without frost damage has been obtained from crop reporters. It must be remembered that these data are of a subjective nature, reflect each reporter's opinion on maturity, and that each report is of a local nature; but combined together they give us what appears to be the only estimate available of the corn that has matured without frost damage in the state of Iowa. These data were used for the percent of corn matured without frost damage (3). Prior to 1945 these data were published in the Iowa Climatological Data.

PROCEDURE AND RESULTS

Prediction from Early Season Temperatures

Indications of a possible "soft corn year" may be obtained early in the season. A wet cool spring usually means late planted corn. Decker (1) found that the silking date was most closely correlated with the temperature for the first 30 days after planting. Reed (5) recognized the importance of early season temperatures in determining the percent of corn matured without frost damage. He also recognized the importance of June temperatures (6) and set a critical limit of between 69 and 70° as the June temperature above which little frost damage would be expected in the fall. However, it would seem that May, July, and August temperatures could not be neglected entirely.

Since Reed's (6) work indicated there would be a very low expectation of frost damage when June had averaged 70° F or greater, only those years from 1926 to 1957 when the mean June temperature was below 70°F

were selected. These included all of the very severe soft corn years since 1926, but did not include 1944, when only 90% of the corn matured, and 1934 and 1943 when 94% matured. The year 1943 had an early freeze, but there was no good explanation why 1944 and 1943 had such a high per cent of corn damaged, except that June was only slightly warmer than the 70°F limit imposed above.

Using these data, correlation coefficients were computed between the percent corn that matured, and the average May, June, July, and August temperatures, and the effective frost date.[3] These correlation coefficients are shown in Table 1. The correlations between percent corn that matured, June temperature and the average frost date were significant at the 1% level. Correlations involving August and July temperatures were either significant or almost significant at the 5% level. The average May temperature was not correlated with the amount of corn matured.

Table 1. Correlation coefficients

Factors	r
Average May temperature and % corn matured	0.05
Average June temperature and % corn matured	0.65**
Average July temperature and % corn matured	0.44
Average August temperature and % corn matured	0.47*
Effective frost date and % corn matured	0.61**

* Significant at 5% level.
** Significant at 1% level.

Since it is desirable to have a prediction as early in the season as possible, a prediction based on June temperatures was attempted. Since one must also determine the effect of frost date on the percent matured, a multiple regression was calculated using June temperature (X_1), effective frost date (X_2) (all dates coded with March 1 as 1, March 2 as 2 etc.) and percent corn matured (Y). The equation computed was

$$\hat{Y} = -227.8 + 3.35X_1 + 0.40X_2 \qquad R = 0.78^{**} \qquad s_{y.12} = 8.1\%. \qquad (1)$$

Without taking into account July and August weather there was considerable deviation of the estimated value from the actual value. The data for 1928 showed a particularly poor relationship.

The simple correlations (multiple correlations were not computed) between percent corn matured and July and August average temperature indicate the accuracy of the prediction might be increased some by inclusion of these temperatures; for an early season prediction, however, only the July temperature would be of use. By August, silking dates are available and these would seem to be the most reliable data to use.

[3] The effective frost date is the average frost date for the state except when a damaging freeze occurs over much of the state, but the rest of the state has no freeze until a much later date. In this case the date of the damaging freeze is used.

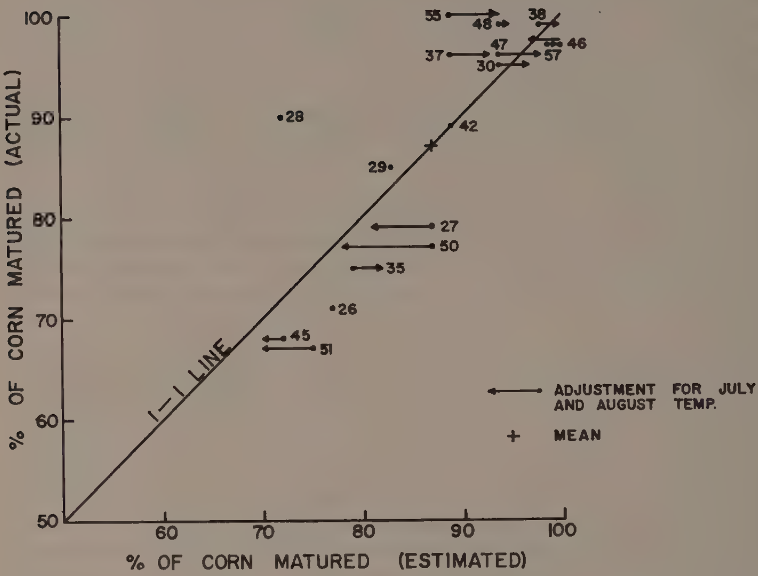

Figure 1. Comparison of estimated and actual corn matured using early season temperatures.

For experimental purposes an arbitrary adjustment was made using these temperatures. These adjustments were as follows.

For each degree the temperature is below normal in July or August, 1% less corn is estimated to mature; for each 2 degrees the temperature is above normal in either month, 1% more corn is estimated to mature.

Using these adjustments, shown by the arrows on Figure 1, the correlation of the predicted amount of corn matured with the actual amount matured was increased to 0.88. Adjustments due to August temperatures were slightly larger than those due to July temperatures.

To check how well this method would do on predicting the amount of corn matured, data for the years 1958, 1959, and 1960 were examined. When equation (1) was used on 1958 data the predicted amount of corn matured was 81.4%. There were no adjustments for July and August temperatures. The actual amount matured was 98%. In the September 3, Iowa Weekly Weather and Crop Bulletin of that year the statement was made "Weekly Crop Reporters indicate that over four-fifths of the corn will be safe from frost damage if frost occurs at normal dates." By the October 7 Bulletin this amount had increased to 95%. Although the weather was favorable for maturing and drying the corn crop, a visual examination of weather records shows no obvious reason for the change. However, the silking data for that year indicated a high percentage of the corn should mature.

The average June temperature in 1959 was above 70°F, so the method could not be tested for that year. In 1960 using equation 1, a predicted amount of 91.9% was computed. This amount, based on freeze date of October 20 (coded value 234), was decreased by 2.9% for July and increased by 0.2% for August temperatures, giving a predicted value of 89.2%. The amount reported mature as of November 1 was 96%.

These data indicate that an early season estimate of possible high amounts of soft corn can be made, but must be used with considerable caution as shown by the wide variations between the predicted and the observed values for 1958 and 1960. Also by using only years when the June temperature averages below 70°F, some years with 5-10% of the corn not reaching maturity may be excluded.

<u>Prediction from Silking Date</u>

Once the corn is silked, a definite stage in its development is established. This provides a further checkpoint for estimating maturity. A preliminary plotting of the data showed that in only two years where the 75% silking date occurred prior to July 30 was there less than 95% of the corn reported mature. In both of these years early freezes occurred. Using those years when silking occurred July 30, or later, the following regression was computed:

$$\hat{Y} = 214.4 - 1.14X_3 + .73X_4 \qquad r = 0.91^{**}, \quad s_{y.34} = 5.1\% \qquad (2)$$

where $\hat{Y}$ = predicted amount of corn matured,
X_3 = average date of silking (coded where July 30 = 152, July 31 = 153, etc.) and
X_4 = days from silking to average freeze date.

This method was also tested for the years 1958-1960. In both 1958 and 1959, the silking date occurred prior to July 30, and equation (2) was not applicable. It would be expected that a high amount of the corn would mature without frost damage, and this was the case. In 1960 the average silking date was August 2, and the first freeze occurred October 19 in part of the state and October 20 over most of the rest of the state. Using these values, the predicted amount of corn matured was 96%, the amount estimated by the crop reporters 96%. As shown by an $s_{y.34}$ of 5.1%, this degree of accuracy would not be expected in many years.

In this paper we have used the average date when the farmer's main field of corn was 75% silked, as shown by the Iowa Phenology Data. In the Iowa Weekly Weather and Crop Bulletin, published jointly by the Agricultural Marketing Service, U.S.D.A., the United States Weather Bureau, U.S.D.C. and the Iowa Department of Agriculture, data are given on the amount of corn silked in Iowa on given dates. An examination of these for recent years shows that the average date of 75% silking, as used in this paper, is equivalent to slightly less than 50% of the corn in Iowa being silked.

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CARBON DIOXIDE FIXING SYSTEMS IN MYCOBACTERIUM PHLEI GROWN IN A SIMPLE CHEMICALLY DEFINED MEDIUM[1]

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SUMMARY. The carboxylating systems in the cell-free extract of Mycobacterium phlei grown in a simple chemically defined medium were investigated. It was found that the extract contains the same enzyme systems which have been found in the extract of the organism grown in a complex medium such as modified Dubos' medium. These enzyme systems are a nucleotide-independent system similar to phosphoenolpyruvic carboxylase and a nucleotide-dependent system similar to oxalacetic carboxylase.

The presence of a synergetic relationship between biotin added and reduced glutathione is indicated.

The participation of sulfhydryl groups for both systems was demonstrated by the sulfhydryl-binding compound, p-chloromercuribenzoate. This inhibition can be completely restored by the addition of reduced glutathione.

The addition of avidin did not affect the nucleotide-independent system, whereas a slight inhibition is observed with a nucleotide-dependent system under the experimental conditions employed.

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In previous papers (Baugh et al., 1959) it was shown that the extract of Mycobacterium phlei grown in a modified Dubos' medium contains at least two different carbon dioxide fixing systems which catalyze the reaction between phosphoenolpyruvate and carbon dioxide to form oxalacetate.

Since the experiments involved synthesis of enzymes, a simple chemically defined medium was needed for comparison with a complex medium such as Dubos' medium. For this comparative investigation a medium containing Tween 80 as a sole source of carbon and ammonium chloride as a sole source of nitrogen was used. The suitability of this medium for the purpose was determined previously by Myoda and Werkman (1959).

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This paper presents the carboxylating systems in the cell-free extract of M. phlei grown in a simple chemically defined medium and describes properties of the reactions.

METHODS AND MATERIALS

Reagents

All reagents were commercial preparations, except the $NaHC^{14}O_3$. $NaHC^{14}O_3$ solution was prepared from $BaC^{14}O_3$ obtained from Oak Ridge National Laboratory according to Hug (1956).

Main commercial preparations used in this experiment and the companies from which obtained are as follows:

Pabst Laboratories, various nucleoside polyphosphates (Na-salts)
California Foundation for Biochemical Research, oxalacetic acid (OAA), phosphoenolpyruvate (tricyclohexylamine salt) (PEP)
Nutritional Biochemicals Corporation, reduced glutathione (GSH), avidin.

Preparation of cell-free extracts

The cultural conditions were the same as in previous papers (Baugh et al., 1959; Myoda and Werkman, 1960). Mycobacterium phlei ATCC 10142 was grown in a medium of the following composition: NH_4Cl, 1.0 g, $Na_2HPO_4 \cdot 12H_2O$, 6.5 g; KH_2PO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.02 g; $CaCl_2$, 0.0005 g; $FeCl_3 \cdot 6H_2O$, 0.0005 g; Tween 80, 5.0 g; and distilled water, 1000 ml. The culture was vigorously aerated by a stream of sterilized air for 48 hours at 37°C. The cells were harvested by centrifugation, washed twice with distilled water, and 5 grams, wet weight, of the cells were suspended in 35 ml of 0.2 M tris buffer pH 7.4. After sonic treatment for 25 minutes then centrifugation, sufficient $MnCl_2$ (1 M) was added to the extract to make a 13% solution and the mixture stirred for 30 minutes at 4°C. The mixture was warmed in a 55°C water bath for a few minutes and centrifuged to remove the precipitated protein. The supernate was dialyzed against 0.002 M tris buffer for 15 hours at 4°C. The dialyzed extracts were stored at -20°C when not used immediately.

Determination of protein

The biuret method of Gornall et al. (1949) was employed. The interference of tris buffer was minimized by analysis of the separated protein precipitate as described by Robinson and Hogden (1940).

Identification of radioactive compounds

Paper chromatography with radioautography or paper ionography with radioautography was used to identify the radioactive compounds. Kodak No-screen medical X-ray film was used for radioautography.

Radioactive oxalacetate formed by the fixation or the exchange reaction was isolated and identified as its 2,4-dinitrophenylhydrazone. The position of radioactive carbon in the hydrazone was determined by the decarboxylation procedure of Bandurski and Greiner (1953).

Assay of radioactivity

Radioactivity was measured with a lead-shielded end-window (mica) Geiger-Muller tube (Tracerlab type TGC2) having a window thickness of 1.8 mg per cm^2. Counts were corrected for background and expressed as counts per minute (c/m). The counting time was selected so as to make the standard deviation within five per cent of the net count.

EXPERIMENTAL

The experimental procedures were the same as in previous papers (Baugh et al., 1959; Myoda and Werkman, 1960). Reactions were carried out in Warburg flasks with two side arms, under an atmosphere of nitrogen. The main chamber contained the substrate, buffer, and any additional cofactors or nucleotides. The contents of the side arms (the extract and radioactive bicarbonate) were tipped into the main chamber after the replacement of the gas phase and temperature equilibration at 31°C.

Table 1 shows the results obtained from a well-dialyzed extract (non-fractionated).

Table 1. $C^{14}O_2$ fixation by a dialyzed extract.[a]

No.	Addition	Activity fixed (c/m/100 µl.)
1	$MnCl_2$, PEP	141
2	$MnCl_2$, PEP, IDP	340
3	$MnCl_2$, PEP, IDP, EDTA	72
4	$MgCl_2$, PEP	0
5	$MgCl_2$, PEP, IDP	34
6	$MgCl_2$, PEP, IDP, EDTA	0

[a]The basal system contained 100 µmoles of tris buffer (pH 7.4), 20 µmoles of $Na_2C^{14}O_3$ (8.8×10^5 c/m), and 0.5 ml of 17-hour dialyzed extract (no endogenous). Compounds added were: PEP, IDP, 3 µmoles each; ions, 5 µmoles; EDTA, 2 µmoles. The total volume was 2.0 ml. The reactions were stopped after an hour by adding 0.2 ml of 50 trichloroacetic acid (TCA). The precipitates formed were centrifuged down, then an aliquot of sample was placed on a glass planchet for counting.

This experiment was carried out with $Na_2C^{14}O_3$ because of the shortage in supply of $NaHC^{14}O_3$ at this time. The specific activity of this sample was lower than that with $NaHC^{14}O_3$.

The expression "no endogenous" indicates no metal ions, no nucleotides, and no residual substrates in the extract employed.

As illustrated in Table 1, the extract fixes carbon dioxide by similar, if not identical, reactions of the extract obtained from cells grown in a complex medium such as Dubos' medium.

With phosphoenolpyruvate, $NaHC^{14}O_3$, and fractionated-extract, oxalacetate containing radioactive carbon in its β-carboxyl group was isolated as an only radioactive compound regardless of the presence or absence

of added nucleotide. In some experiments two more minor radioactive products were detected on the radioautogram. These compounds have not been identified as yet because of their small radioactivities. This is thought to be due to the impurity of the extracts which may contain some other enzyme systems such as transaminases.

With non-radioactive oxalacetate the radioactive oxalacetate was isolated as an only radioactive compound from the reaction mixture. These results indicate the presence of at least two enzyme systems, i.e. phosphoenolpyruvic and oxalacetic carboxylases, in the extract of Mycobacterium phlei grown in a simple chemically defined medium. These two enzymes catalyze the following reactions respectively:

$$\text{Phosphoenolpyruvate} + CO_2 \rightarrow \text{Oxalacetate} + \text{Orthophosphate} \quad (1)$$
$$\text{Phosphoenolpyruvate} + CO_2 + \text{IDP} \rightleftarrows \text{Oxalacetate} + \text{ITP} \quad (2)$$

where IDP and ITP represent inosine di- and triphosphate respectively.

Nucleotide specificity and its optimal concentration

Since the extract can fix radioactive carbon dioxide according to equation 2, the effect of other nucleotides was determined. The results are shown in Table 2.

Table 2. Nucleotide specificity.[a]

No.	Nucleotide	Activity fixed (c/m/100 µl.)
1	None	180
2	IDP	454
3	UDP	406
4	CDP	274
5	ADP	274
6	GDP	824

[a]The basal system contained 100 µmoles of tris buffer (pH 7.4), 2 µmoles of PEP, 2.5 µmoles of $NaHC^{14}O_3$ (1×10^7 c/m), and 5 µmoles of $MnCl_2$. Two-tenths of a ml of manganese-fractionated, 16-hour dialyzed extract (0.8 mg protein/ml and no endogenous) were used. Two µmoles of a nucleotide were used. The total volume was 2.0 ml. The other conditions were the same as in Table 1.

Inosine and quanosine diphosphate stimulate the fixation. Uridine diphosphate is substantially as effective as inosine diphosphate.

The optimal concentration of inosine diphosphate is shown in Figure 1. The rate of fixation is maximal when the relationship between phosphoenolpyruvate and inosine diphosphate is equimolar. These results are in agreement with a former case (Myoda and Werkman, 1960).

According to Tchen and Vennesland (1955) the phosphoenolpyruvic carboxylase loses its activity after dialysis against collidine buffer, whereas the oxalacetic carboxylase is intact. The manganese-fractionated extracts were dialyzed against collidine buffer (0.01 M, pH 7.4), then the activity was determined. This treatment caused complete inactivation of both enzyme systems, i.e. nucleotide-independent and dependent systems.

Effects of glutathione and biotin

In a previous report (Myoda and Werkman, 1960) it was found that the addition of reduced glutathione, in some cases, stimulated the rate of fixation of carbon dioxide and that the effect was significant with magnesium ions.

Biotin has been considered to have a role in the fixation reaction. Since the chemically defined medium used in this study contains no organic compound except Tween 80, the effects of biotin and glutathione were determined (Table 3).

Table 3. Effects of glutathione and biotin.[a]

No.	Addition	Activity fixed (c/m/100 µl.)
1	None	283
2	GSH	234
3	GSH, Biotin	306
4	Biotin	244
5	IDP	1024
6	IDP, GSH	1476
7	IDP, GSH, Biotin	1438
8	IDP, Biotin	888

[a] The basal system contained 100 µmoles of tris buffer (pH 7.4), 3 µmoles of PEP, and 2.5 µmoles of $NaHC^{14}O_3$ (1×10^7 c/m). Compounds used were: GSH, 5 µmoles, biotin 25 µg, IDP, 3 µmoles. Two-tenths ml of the manganese-fractionated, 16-hour dialyzed extract (0.8 mg protein/ml and no endogenous) were used. The other conditions were the same as in Table 1.

The addition of biotin at pH 7.0 seems to cause a slight inhibition of fixation of carbon dioxide. The addition of reduced glutathione stimulates the rate of a nucleotide-dependent fixation. The addition of both glutathione and biotin appears to have a synergistic effect on the nucleotide-independent system.

Effect of p-chloromercuribenzoate

In order to determine the necessity of sulfhydryl groups for the activity of the enzyme systems the effect of a sulfhydryl-binding compound, p-chloromercuribenzoate (p-CMB), was determined. The results are summarized in Table 4.

The addition of the sulfhydryl-binding compound caused complete inactivation of the fixation reactions. The addition of reduced glutathione reversed the inhibition. When large amounts of glutathione (30 µmoles) were added to the systems to which p-chloromercuribenzoate (0.125 µmole) was added, the complete recovery of the reactions, or rather increased fixation, resulted. The radioactivities of 200 and 1844 counts per minute per 100 µl were fixed by the nucleotide-independent and dependent systems respectively. Reasons for this increase in activity are not known. These results indicate the necessity of sulfhydryl groups for activity of the enzyme.

Table 4. Effect of p-chloromercuribenzoate on the fixation.[a]

No.	Addition	Activity fixed (c/m/100 µl.)
1	None	544
2	p-CMB	0
3	GSH, p-CMB	130
4	GSH	398
5	IDP	1172
6	IDP, p-CMB	0
7	IDP, p-CMB, GSH	200
8	IDP, GSH	950

[a] The basal system contained 100 µmoles of tris buffer (pH 7.4) 0.2 ml of manganese-fractionated, 16-hour dialyzed extracts (0.8 mg protein/ml and no endogenous), 5 µmoles of $MnCl_2$, 6 µmoles of PEP, and 5 µmoles of $NaHC^{14}O_3$ (2 x 10^7 c/m). IDP added was 6 µmoles and GSH added was 5 µmoles. p-CMB (0.125 µmole) was placed in a side-arm with the extracts during the gassing. The other conditions were the same as in Table 1.

Effect of avidin

In the previous section it was found that the additional biotin does not have a significant effect on the fixations. An attempt was made to determine whether or not the extracts contain enough biotin for the activity using a biotin-binding compound, avidin. Since avidin is a water-insoluble protein, ammonium sulfate or physiological saline solution was used as a solvent. One mg of avidin (2.5 units) was dissolved into 1 ml of 50% saturated ammonium sulfate solution. The solution was mixed with 1 ml of the extract (mangenese-fractionated and 16-hour dialyzed) then incubated for an hour at room temperature. As a control 1 ml of 50% saturated ammonium sulfate solution was added to 1 ml of the extract and treated in the same way. Since the excess of ammonium sulfate inhibits the fixation, those reaction mixtures were dialyzed for 7 hours. As illustrated in Table 5, the addition of avidin does not affect the nucleotide-independent fixation but does the nucleotide-dependent fixation.

Table 5. Effect of avidin.[a]

No.	Addition	Activity fixed (c/m/100 µl.)
1	None	98
2	Avidin	98
3	IDP	494
4	IDP, Avidin	258

[a] The basal system contained 100 µmoles of tris buffer (pH 7.4), 2.5 µmoles of $NaHC^{14}O_3$ (1 x 10^7 c/m), 3 µmoles of PEP, 5 µmoles of $MnCl_2$, 5 µmoles of GSH, and 0.4 ml of the extracts (see text). IDP added was 3 µmoles. The other conditions were the same as in Table 1.

When avidin was dissolved in physiological saline solution, no dialysis was required because of no inhibitory action of saline solution itself on the fixation reactions. It was found, however, that no significant effect of avidin was demonstrated (Table 6).

Table 6. Effect of avidin in physiological saline solution.[a]

No.	Avidin added (μg)	Activity fixed (c/m/100 μl)
1	None	566
2	62.5	536
3	125.0	548
4	250.0	504
5	375.0	478
6	500.0	504

[a] The conditions were the same as in Table 5, except different amount of avidin in physiological saline solution was used. The system contained 3 μmoles of IDP. Avidin was reacted with the extracts for an hour at room temperature.

DISCUSSION

The comparative study, between the extract obtained from cells grown in a complex medium such as a modified Dubos' medium and those grown in a simple chemically defined medium, has been made with respect to the synthesis of the carboxylation enzymes. The present study indicates that M. phlei can produce the same carboxylating enzyme systems regardless of the medium used. Results are in agreement. Both systems, i.e. nucleotide-independent and dependent systems, require a divalent metal ion for their activities. These enzyme systems catalyze the following reactions respectively.

$$\text{Phosphoenolpyruvate} + CO_2 \rightarrow \text{Oxalacetate} + \text{Orthophosphate}$$

$$\text{Phosphoenolpyruvate} + CO_2 + \text{ODP} \rightleftarrows \text{Oxalacetate} + \text{ITP}$$

The rate of fixation of carbon dioxide by a nucleotide-dependent system is greater with guanosine and inosine polyphosphate, especially the former. Uridine polyphosphate is nearly as active as inosine polyphosphate. Although the results are not shown, the experiments were carried out to determine whether or not this effect of other nucleotides is due to the presence of nucleoside diphosphokinase. All attempts to demonstrate conclusive evidence for the presence of the enzyme in the extract of M. phlei have been unsuccessful. The optimal concentration of IDP is found to be equimolar, with the substrate, phosphoenolpyruvate.

Blanchard et al. (1950) suggested that biotin may be involved in the synthesis of the specific enzymes that mediate the fixation reaction. Since the chemically defined medium used in this study does not contain any organic compound except Tween 80, if biotin is involved, it is a

logical assumption that M. phlei can produce biotin in a simple chemically defined medium. Pope and Smith (1946) reported the ability of mycobacteria to synthesize biotin and other vitamins on a simple medium. In a study of growth inhibition Pope and Smith (1950) concluded that p-aminobenzoic acid and biotin are essential factors in the metabolism of the tubercle bacillus.

Biotin has been thought to play a role in the fixation of carbon dioxide. It was first postulated by Burk and Winzler (1943) that biotin might function as a coenzyme of biological carbon dioxide transfer. The studies of Lichstein and Umbriet (1947), Shive and Rogers (1947), Lardy et al. (1947) and Ochoa et al. (1947) suggested a role for biotin in the Wood-Werkman reaction. Wessman and Werkman (1950) found conclusive evidence that biotin functioned as a coenzyme in the fixation. The authors found that avidin prevented the exchange reaction between oxalacetic acid and $NaHC^{13}O_3$ and that the addition of adequate biotin resulted in a return to normal fixation. Schaefer et al. (1955) and Schaefer (1957) reported the presence of a relationship between carbon dioxide requirement and biotin requirement of the tubercle bacillus.

The present study shows that additional biotin has a rather slight inhibitory action on the fixation. However, the results indicate a synergistic relationship between biotin and reduced glutathione on a nucleotide-independent system.

Avidin, biotin-binding compound, was used to determine the participation of biotin in the fixation. The results indicated that avidin does not inhibit the nucleotide-independent system but does inhibit the nucleotide-dependent system. Complete inhibition has not been obtained. This could be due to the unfavorable condition for the reaction between avidin and biotin, or a presence of biotin-like substance which is unaffected by avidin. Burk and Winzler (1943) isolated this type of compound from urine of several animals. The role of biotin in the fixation is not well understood.

The necessity of the sulfhydryl group for the fixation reactions was shown by using a sulfhydryl-binding compound, p-chloromercuribenzoate. Even a small amount of the inhibitor inhibits both reactions, i.e. nucleotide-dependent and -independent, completely. The addition of reduced glutathione restored the reactions completely. The optimal concentration of added glutathione seems to vary with the freshness of the extracts and with the preparation of extracts, (Myoda and Werkman, 1960).

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TEN NEW SPECIES OF PHYTOCORIS FROM NORTH AMERICA

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ABSTRACT. By the close of 1958 the genus Phytocoris numbered 307 species known from the world; counting the present contribution 159 are known from the United States. New Phytocoris are utahensis, stitti, yuma, angustatus, miniatus, and rubroornatus from Arizona; viridescens from Colorado; texanus from Texas; ephedrae from Arizona and Texas; albidopictus from Arizona, California and Texas. Illustrations are given of the genital segment showing the male claspers. A full page drawing of Phytocoris heidemanni Reuter is made from the unique female type specimen.

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The genus Phytocoris includes some 307 named species as recorded at the close of the year 1958, making it the largest genus in the family Miridae. Of this number 149 species are recorded from America north of Mexico. With the present contribution the number is raised to 159.

The best characters for distinguishing the species are found in the genital segment, especially the claspers and structure of the vesica. Other important characters are found in form of head and antennae, particularly the relative lengths, thickness and form of the segments; also bristles, pubescence, and color areas, including membrane of hemelytra. After working over the material on hand the following species are described as new.

Phytocoris utahensis new species.

Allied to conspurcatus Kngt. and runs in the couplet with this species in my key to Phytocoris (Miridae of Illinois, 1941), but differs in the relatively longer first annual segment, the length of which greatly exceeds the width of head across the eyes; also the pale band just beyond middle of second antennal segment, is more diffuse, not sharply defined.

Male. Length 6.4 mm, width across hemelytra 2.2 mm. Head: width 1.04 mm, vertex .34 mm, dark lines and spots similar to conspurcatus. Antennal segment I, length 1.4 mm, white with a reticulate pattern of black, making the white appear as spots from which erect white bristles arise, the black areas thickly set with shorter, recumbent black hairs; II, 2.72 mm, fuscous to black, a white annulus at base, a second cream colored band beginning at middle and extending for a space .27 mm, the

remainder blackish, finely pubescent; III, 1.7 mm, black, pallid at base, also a cream band just beyond middle; IV, 1.2 mm, black. Rostrum, length 2.72 mm, extending upon eighth ventral segment. Pronotum: length .98 mm, width at base 1.73 mm, form, color and pubescence very similar to that of conspurcatus; perhaps the black scales more abundant, thickly grouped at six points along the subbasal crenulate black band. Scutellum quite similar to that of conspurcatus but the blackish color concentrated along the median line. Hemelytra and dorsal surface of pronotum thickly clothed with short pubescent hairs, intermixed with black scalelike hairs and small patches of dense, white woolly hairs. Membrane marbled with fuscous, the dark color composed of dots and reticulations, leaving very little solid shading along the veins. Legs colored much like conspurcatus, but the white bands on tibiae broader than the dark bands. Genital segment distinctive, a slender tubercle each side arising from the segment wall just above base of clasper, much as in conspurcatus, but the form of right clasper differs as shown in Figure 2.

Female. Length 6.1 mm, width 2.2 mm. Head: width 1.05 mm, vertex .41 mm. Pronotum: width at base, 1.73 mm. Antennae: segment I, length 1.53 mm; II, 2.78 mm; III, 1.7 mm; IV, broken. Coloration and pubescence similar to the male. The species is best distinguished by the longer first antennal segment.

Holotype: ♂ Aug. 15, 1929, at light trap, Richfield, Utah (E.W. Davis); author's collection. Allotype: ♀ July 8, 1929, at trap light, Richfield, Utah (E.W. Davis). Paratype: ♂, taken with the allotype. ♂ July 15, 1929, at trap light, Richfield, Utah (E.W. Davis).

Phytocoris calli Kngt. is closely related to utahensis but may be distinguished by the black antennal segment III which is narrowly pale only at base.

Phytocoris stitti new species.

Allied to plenus Van D., but distinguished by the uniformly fuscous brown second antennal segment which is narrowly pale at base, also by the shorter spines on Segment I which in length scarcely exceed diameter of the segment.

Male. Length 7.6 mm, width 2.5 mm. Head: width 1.12 mm, vertex .37 mm, flat; frons tumid, creamy white, marked with oblique fuscous lines, tylus and lora faintly marked with reddish. Rostrum, length 2.5 mm, reaching upon third ventral segment, pallid, apical segment fuscous. Antennae: segment I, length 1.53 mm, base and apex thicker than the more slender portion of apical half; pallid white, marked with fuscous reticulations which outline small and large white spots, clothed with rather short, suberect dark pubescent hairs, the glabrous spots bearing short, erect, dusky bristles which in length about equal thickness of segment; II, 3.3 mm, cylindrical, uniformly fuscous brown, narrowly pale at base, clothed with very fine short pubescence; III, 1.53 mm, blackish; IV, .78 mm, black. Pronotum: length 1.05 mm, width at base 1.9 mm; disk and calli covered with blotches and reticulate marks of black over tan background, a submarginal black line shows each side of middle but the basal angles are yellowish; calli marked with reticulate black lines, inner angles pallid; collar nearly flat, a few short dark

Figure 1. Phytocoris heidemanni Reuter. Drawing made from the unique female type in the United States National Museum.

hairs above, yellowish, a black ray crosses behind eye; propleura pallid below, dorsal margin fuscous, a dark fuscous ray crosses middle of coxal cleft and flares posteriorly. Mesoscutum dull black, a tan spot each side opposite basal angle of scutellum. Scutellum fuscous to black, apex broadly, basal angles, and narrow but distinct median line, yellowish or tan. Pronotal disk, scutellum and hemelytra sparsely clothed with short, black pubescences, and intermixed with golden and silvery, sericeous pubescence.

Hemelytra shaded with dark fuscous, the dark color broken by paler blotches and dots of tan and pale areas, cuneus only slightly darker on apex.

Membrane pallid, rather evenly sprinkled with reticulate marks and dots of fuscous, area around apex of cuneus and a margin spot just beyond, clear of dots. Legs pallid to yellowish, all femora covered rather evenly with reticulate fuscous lines, leaving both small and large pale spots within the network; both front and middle tibiae have four pale annuli, but the fuscous bands broken by many small pale dots; tibial bristles rather short, their length scarcely exceeding diameter of tibia. Venter pale yellowish, sides and the genital segment shaded with fuscous; genital segment and claspers distinctive, a broad blunt tubercle formed on angle of segment wall above base of left clasper, also a tiny smooth tubercle set above base of the small right clasper (Figure 2).

Holotype: ♂ April 9, 1942, Tucson, Arizona (Loyd L. Stitt); author's collection. Paratypes: 2 ♂♂ April 10, 1942, Tucson, Arizona (Loyd L. Stitt). Named for the collector, Mr. Loyd Stitt, who collected many rare and new species of Miridae while working on the problem of alfalfa seed destruction by Lygus bugs, primarily Lygus hesperus Kngt.

Phytocoris albidopictus new species.

Allied to pulchricollis Van D. but differs in the shorter first antennal segment which barely exceeds width of head across eyes and in having only three white spots on dorsal surface of segment I.

Male. Length 4.1 mm, width across hemelytra 1.46 mm. Head: width .82 mm, vertex .41 mm; vertex, frons and basal half of tylus, black, apical half of tylus and other facial parts white. Rostrum, length 2.3 mm, extending to seventh ventral segment, white, apical segment fuscous. Antennae: segment I, length .85 mm, slightly thicker on basal half, black, three large white spots on dorsal surface, clothed with suberect black hairs, also one or two white bristles arising from each white spot; II, 2.24 mm, slender, cylindrical, blackish, annulus at base and one just beyond middle, white, between are two white half bands on dorsal aspect, clothed with very short pubescence; III, 1.29 mm, black, a white band at base; IV, .95 mm, black. Pronotum: length .78 mm, width at base 1.36 mm, disk brownish black, median line except posterior third, undulating basal margin, narrow lateral margins and propleura, white, a black line extending back from top of coxal cleft and bordering the white margin of the disk; the disk clothed with a mixture of black and white sericeous pubescence, the white basal margin bearing white woolly pubescence, collar with several black bristles. Mesoscutum black, bearing sericeous white pubescence, mesosternum black. Scutellum moderately convex, apical area more flat; black, apical third and basal

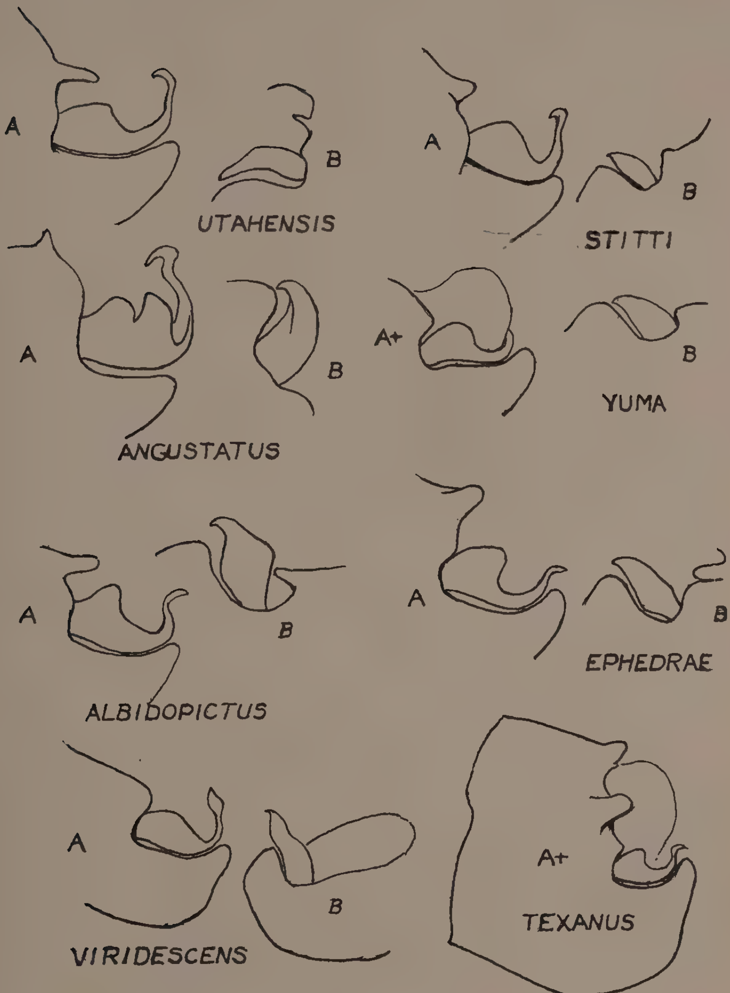

Figure 2. Phytocoris species, name of each species shown with genital structures.

A Left clasper in situ with outline of segment wall.
A+ Same as A but with membrane canopy shown covering genital chamber as in life.
B Right clasper in situ with outline of segment wall.

angles white, clothed with appressed white pubescence. Hemelytra opaque white, middle third of claval vein, inner apical angle of corium and extending upon paracuneus, spot beyond middle and larger one on apex of embolium, apex of cuneus and spots on disk, brownish black, clothed with appressed sericeous white pubescence; blackish hairs on the dark areas, apex of clavus and tip of cuneus with tufts of woolly black hairs. Membrane opaque white, reticulated with black, fading out on discal area and two marginal spots beyond cuneus, a dark ray extending back from apex of areoles, basal half of veins dark. Legs white, front femora with row of dots above and black line on posterior aspect, front tibiae black on base and apex with three white annuli between, tarsi black; middle femora black on apical area, joined above with black line on apical half, tibiae white, bearing just a few small dark marks; hind femora white, apical half with reticulate pattern of brownish black marks, becoming solid black above on apical half; tibiae white, with many large and small black dots and marks, set with many black and white erect bristles. Venter black, ventral aspect white, the adjoining dark color with two or three white spots per segment, genital segment black, each side with three or four white spots near base of clasper; claspers and tubercles distinctive, a tubercle arising from segment wall at the angle formed above base of left clasper, a slightly smaller tubercle arising from edge of segment just above base of the small right clasper (Figure 2).

Female. Length 3.5 mm, width 1.53 mm. Head: width .85 mm, vertex .44 mm. Antennae: segment I, .98 mm; II, 2.2 mm; III, 1.5 mm; IV, 1.02 mm. Pronotum: length .75 mm, width at base 1.32 mm. Slightly larger than the male but color pattern, pubescence and essential measurements very similar to the male.

Holotype: ♂ Sept. 2, 1928, alt. 3300 ft., Rincon Mts., Arizong (Andrew A. Nichol); author's collection. Allotype: ♀ July 13, 1917, at light, Lordsburg, New Mexico (H.H. Knight). Paratypes: 2 ♂ Sept. 9, 1928, at light, Tucson, Arizona (A.A. Nichol). ♂ May 12, ♂ July 19, 1929, Tucson, Arizona (E.D. Ball). TEXAS: ♀ May 23, ♀ May 29, at trap light, Castolon (F.F. Bibby). CALIFORNIA: ♂, 3 ♀♀ June 6, 1930, Mojave (R.L. Usinger).

Phytocoris ephedrae new species.

Allied to ventralis Van D. but differs in the antennae, in the sharp heavy black longitudinal line on each side of the abdomen, but without a black line on propleura; form of genital segment and claspers distinctive (Figure 2).

Male. Length 4.1 mm, width 1.36 mm. Head: width .82 mm, vertex .37 mm, white, a pair of small black dots, one each side near eye, eyes reddish brown. Rostrum: length 1.80 mm, reaching upon basal half of genital segment, pallid, fuscous on apical half. Antennae: segment I, length .68 mm, pallid, thickly clothed with pale hair on ventral surface, more sparsely set above, a few pallid bristles that barely stand out beyond the suberect hairs; II, 1.70 mm, cylindrical, pallid, pale fuscous near apex, clothed with fine short pubescence; III, 1.05 mm, fuscous, narrowly pallid at base; IV, .54 mm, fuscous. Pronotum: length .65 mm, width at base 1.19 mm, white, the collar, shading on

calli, median line of disk, and spot at top of coxal cleft, purplish brown; basal submargin of disk provided with an undulating dark brown line, this having two raised points each side of middle that fit into curves of the dark line; basal edge of disk and the median line bearing white sericeous pubescence, the discal area having simple black pubescence, while behind the calli and lateral areas of disk, intermixed with black scalelike hairs; propleura white, devoid of black lines as found in ventralis Van D. Scutellum white, a fuscous spot each side before apex, median triangle at base and the mesoscutum brownish black; clothed with appressed white sericeous pubescence, a few black scalelike hairs on apical half. Hemelytra opaque white, clothed with simple pubescence, but intermixed with white sericeous pubescence and sprinkled with black scalelike hairs; tufts of black scales at tip of clavus, tip of cuneus and spot midway along inner edge. Membrane white, thickly sprinkled with reticulate black marks and dots, two small clear spots along margin behind cuneus. Legs white, apical third of femora shaded with fuscous but broken by pale spots; tibiae pallid, hind pair with small dark spots near base, sparsely set with long white bristles, length of some about twice the diameter of the tibia, tarsi pale. Venter white, segments 2-8 with lateral deep black line extending forward and crossing the pleura; in ventralis a broad area on sides of venter is covered with black. Genital segment white, sides shaded with fuscous, claspers distinctive, segment wall bearing a thick, rather broad tubercle projecting from angle of segment wall above base of left clasper, also a nearly obsolete small tubercle just before base of the small right clasper (Figure 2).

Female. Length 4.2 mm, width 1.42 mm. Head: width .82 mm, vertex .41 mm. Antennae: segment I, length .74 mm; II, 1.98 mm; III, 1.2 mm; IV, broken. Pronotum: length .48 mm, width at base 1.20 mm. Very similar to the male in form, pubescence and coloration; this specimen with black areas intensified, a bit of infuscation appearing on middle of claval vein, radius and cubitus, membrane reticulations nearly black; the black lateral line on venter, sharp and distinctive as in the male.

Holotype: ♂ July 15, 1917, Bowie, Arizona (H.H. Knight); author's collection. Allotype: ♀ Sept. 9, 1928, Tucson, Arizona (A.A. Nichol). Paratypes: ♂, taken with the allotype, found breeding on Ephedra sp. by Mr. Nichol. 2 ♂♂ May 20, 1928, alt. 5000 ft., Empire Mts. Arizona (A. A. Nichol) breeding on Ephedra sp. ♀ May 12, 1929, ♀ July 19, 1929, Tucson, Arizona (E.D. Ball). TEXAS: ♂ May 23, 1928, Castolon (F.F. Bibby); 2 ♂♂ Sept. 28, 1929, Presidio (W.L. Owens).

Phytocoris yuma new species.

General aspect suggestive of strigosus Kngt., especially in the fuscous lines on hemelytra but size larger, the pale band at middle of second antennal segment not sharply defined; male genital segment without tubercle above base of left clasper.

Male. Length 5.8 mm, width 1.9 mm. Head: width 1.05 mm, vertex .476 mm; white, four marks on vertex, apex and oblique lines each side of frons, V-shaped mark on base and band across middle of tylus, dorsal margin of lora, base of juga, and eyes, brownish black. Rostrum, length 2.5 mm, reaching to middle of seventh ventral segment, pallid, fuscous

on apical half. Antennae: segment I, length 1.40 mm, black, anterior aspect with several large and small white glabrous spots, an erect white bristle arising from each of the larger spots, clothed with both white and black pubescent hairs; II, 2.82 mm, fuscous to black, a white band at base, a pale or cream band beginning at middle grading into fuscous on apical half, not sharply defined, clothed with fine, short yellowish pubescence; III, 1.9 mm, black, white band at base; IV, 1.3 mm, black. Pronotum: length .92 mm, width at base 1.70 mm, disk grayish white, with crenate subbasal black line, clothed with prominent black pubescence; calli mottled with fuscous, outer angles blackish; propleura white, a black ray across middle of coxal cleft and extending posteriorly, dorsal margin not delimited by lines. Scutellum convex, apical area more flattened, creamy white, median line delimited by irregular fuscous marks each side, apical half more pallid, a strong fuscous patch each side extending to claval margin.

Hemelytra pallid to dusky white, claval vein and a broken line parallel with claval suture, blackish; corium bordering clavus and extending to cover inner half of paracuneus, radial vein, spots on embolium, and small dots on inner half of cuneus, blackish. Membrane and veins milky white, vein about smaller areole and an arcuate ray extending back behind areoles, brownish black; remaining areas filled with dots and reticulate fuscous marks, two small clear areas along margin behind tip of cuneus. Legs white to creamy white, front femora with dorsal and posterior, irregular and broken, longitudinal blackish lines; front tibiae white, annulus on apex, middle and at middle of basal half, black, spines and hairs take the color of surface from which they arise, tarsi fuscous; middle femora with black marks on apical balf only, tibiae with four black marks or incomplete annuli, counting the narrowly black apex; hind femora strongly thickened on basal half, tapering to rather slender on apical one-fourth, white, apical half covered with black reticulations which are arranged on anterior face in irregular, oblique patches and lines, the black areas broken by white dots; clothed with inconspicuous white pubescence; a wide irregular section near base, another at middle, and a few irregular dots and marks on apical half, black. Venter largely white, sides with one longitudinal black line, above and below this line, marked with short dashes and dots of black; sides of genital segment shaded with fuscous, elsewhere with fuscous dots and reticulations; form of genital segment and claspers distinctive, devoid of tubercles near base of claspers (Figure 2).

Holotype: ♂ Nov. 4, 1939, Yuma Co., Arizona (Loyd L. Stitt); author's collection. Paratypes: 11 ♂♂, taken with the type at trap light. The absence of females would indicate they may be brachypterous, as is true of several known desert species of *Phytocoris*. Named after the Yuma Indians, and from whom the county takes its name.

Phytocoris miniatus new species.

Allied to *breviusculus* Reut., but smaller, vertex broader as compared with width of head, also without tubercle above base of left genital clasper; venter, cuneus, embolium and outer apical angle of corium, distinctly red.

Male. Length 3.3 mm, width 1.32 mm. Head: width .75 mm, vertex

.31 mm, frons marked with reddish brown, lower face marked with red much like breviusculus. Rostrum, length 1.49 mm, reaching upon base of genital segment. Antennae: segment I, length .54 mm, pallid, apex, blotch at middle and one on basal half, reddish brown, anterior face with a few short bristles; II, 1.36 mm, cylindrical, light reddish brown, pale at base, clothed with fine short pubescence; III, .98 mm, fuscous; IV, .58 mm, fuscous. Pronotum: length .64 mm, width at base 1.20 mm; yellowish to reddish brown, collar greenish white, a small spot of red each side extending upon calli, a larger red spot behind each eye, propleura pallid on ventral half. Dorsum clothed with yellowish to brown simple pubescence, sparsely intermixed with silvery sericeous pubescence. Scutellum reddish, irrorate with pale spots, median line and apical area pallid. Hemelytra suggestive of breviusculus, with strong fuscous shading on corium, but outer apical half of corium and the embolium distinctly red; cuneus red, three small yellow dots on outer edge, apex, spot on inner edge, and the paracuneus, fuscous. Membrane dark fuscous, sprinkled with dots and reticulations, large spot on margin behind cuneus, a second smaller one beyond, milky white. Legs pallid to white, marked with reddish brown reticulations; tibiae rather indistinctly banded with brownish. Venter red to brownish red, genital segment more or less pallid on middle of ventral surface; genital segment differs from breviusculus in the absence of a tubercle above base of left clasper, also in form of the claspers.

Female. Length 3.5 mm, width 1.53 mm. Head: width .76 mm, vertex .37 mm. Antennae: segment I, length .58 mm; II, 1.49 mm; III, .92 mm; IV, .68 mm. More robust than the male but very similar in coloration, pubescence and characters of head and antennae.

Holotype: ♂ May 12, 1929, Tucson, Arizona (E. D. Ball); author's collection. Allotype: ♀, same data as the type. Paratypes: 1 ♂, 4 ♀♀ taken with the type.

Phytocoris texanus new species.

Related to roseus Uhler but size smaller; differs in form of head, eyes very small, placed far forward away from base, reminding one of the genus Macrolophus in this respect; antennae not banded with black, segment I deep red and with white spots.

Male. Length 5.6 mm, width 1.8 mm. Head: width .84 mm, vertex .44 mm, relatively wide; eyes small, height .38 mm, space between eye and collar, .14 mm; yellow, unmarked except for red ray behind eye, frons clothed with prominent pale pubescence. Rostrum, length 3.0 mm, reaching upon the eighth ventral segment, yellowish, apex blackish. Antennae: segment I, length 1.46 mm, slightly thicker near base and at apex, deep red, about 10 white glabrous spots on anterior aspect, each spot bearing an erect white bristle which in length about equal to diameter of segment, the red surface bearing recumbent black pubescence; II, 2.55 mm, cylindrical, yellowish, reddish near base; III, 1.63 mm, yellowish; IV, 1.09 mm, fuscous. Pronotum: length .88 mm, width at base 1.46 mm; collar rather flat above, bearing bristle hairs, anterior angles not well developed, top of coxal cleft visible from above; basal half of disk red. Mesoscutum red, broadly exposed. Scutellum moderately convex, uniformly yellow. Hemelytra yellow, inner apical margin

of corium, paracuneus, apex and inner margin of cuneus bright red; clothed with suberect simple yellowish pubescence. Membrane dark fuscous, finely dotted with pale marks, a spot behind apex of cuneus and lunate marginal spot behind, clear, veins pale. Legs pale to yellowish, apical half of femora deep red, spotted with white, hind femora with a larger, saddle-shaped, dorsal white mark on apical one-fourth, pubescence rather short and inconspicuous; tibiae pallid, basal one-fourth reddish and spotted with pale, spines yellow, length exceeding diameter of tibia, tarsi yellowish. Venter uniformly yellow; genital segment distinctive, a large, wide, chisel-shaped tubercle well above and anterior to base of left clasper; a second rather blunt, heavy tubercle on median line above at edge of genital chamber, and a small tubercle on right side anterior to the small right clasper; form of claspers also distinctive of the species (Figure 2).

Holotype: ♂ June 25, 1917, Victoria, Texas (H. H. Knight); author's collection.

Phytocoris rubroornatus new species.

Allied to pulchellus Kngt., about the same size and color, but distinguished by the thicker first antennal segment, by having four tumid points on basal submargin of pronotal disk, and by the deep rose red color of the cuneus and paracuneus.

Female. Length 1.52 mm, width 1.66 mm. Head: width 1.86 mm, vertex .40 mm, frons more tumid and deeply impressed on suture at base of tylus; yellow, basal half of lora, around antennal socket, and oblique striate lines on sides of frons, bright red. Antennae; segment I, length 1.12 mm, thickness .13 mm, orange yellow, touch of red near base, clothed with recumbent yellowish pubescence, sparsely set on anterior face with 8-10 erect pale bristles; II, 2.2 mm, slender, pale yellowish brown; III, broken. Rostrum, length 2.14 mm, extending beyond hind coxae to near base of ovipositor. Pronotum: length .68 mm, width at base 1.26 mm, basal submargin of disk with four raised tumid points, disk bearing simple golden pubescence as upon the head, collar rather flat, set with several yellowish bristles; color bright rose red upon disk, yellow between calli and at top of collar, propleura and xyphus creamy white, mesosternum largely red; mesoscutum red, scutellum creamy white, moderately convex. Hemelytra uniformly opaque, creamy white, cuneus and paracuneus deep rose red, clothed only with simple, short, silvery pubescence. Membrane fuscous, with white to pallid minute spots and reticulations, the paler areas near cuneus and veins, filled with fuscous reticulations, veins bright red. Venter creamy white, genital segments red. Coxae creamy white, front legs dusky orange, basal third of femora pallid; hind femora rose red, sprinkled with small cream spots on apical half; hind tibiae white, a broad basal band bright red, clothed with pallid simple pubescence, and more sparsely set with yellowish bristles which in length about equal thickness of tibia, tarsi yellowish brown.

Holotype: ♂ Aug. 15, 1930, Williams, Arizona (E.D. Ball); author's collection. Our guess is this species will be found to feed on oak as is true of pulchellus and some related species.

Phytocoris viridescens new species.

Closely allied to interspersus Uhler, having two small subapical black spots on scutellum, but differs in having the scutellum distinctly less convex, in wider vertex, and in the quite uniform subopaque pale blue-green color of the hemelytra; genital claspers distinctive.

Male. Length 6.6 mm, width 2.2 mm. Head: width .95 mm, vertex .34 mm; creamy white, a touch of yellow on vertex and frons. Rostrum, length 2.44 mm, reaching upon seventh ventral segment, pallid, apex brownish. Antennae: segment I, length 1.20 mm, slightly more slender on apical half, rather uniformly pallid, somewhat yellowish on basal half but without definite marks, sparsely set with suberect pale hairs, and a few pallid bristles, the length of bristles about equal to diameter of segment; II, 2.42 mm, cylindrical, pallid white, tinged with yellow on apical half; III, 1.40 mm, yellowish, apical half dusky; IV, 1.02 mm, pale to dusky. Pronotum: length .82 mm, width at base 1.50 mm; pallid to white tinged with yellow but no definite pattern of marks visible. Mesoscutum yellowish. Scutellum moderately convex, not subinflated as found in interspersus, pale greenish yellow, a pair of round black dots, one each side of apex. Hemelytra pale blue-green over an opaque white ground color, a reticulate pattern of dusky green over this. Membrane pallid white, sparsely sprinkled with dusky dots, veins greenish white. Legs pale to white, hind femora with fine reddish brown reticulations on apical half; tibiae unmarked, spines and pubescence pallid to clear, tarsi dusky yellow. Venter pallid to greenish, genital segment more yellowish on basal half; claspers distinctive, close to interspersus but different (Figure 2).

Female. Length 5.9 mm, width 2.1 mm. Head: width .95 mm, vertex .42 mm. Antennae: segment I, 1.2 mm; II, 2.24 mm; III, 1.23 mm; IV, broken. Pronotum: length .85 mm, width at base 1.53 mm. Very similar to the male in form, color, and pubescence.

Holotype: ♂ Aug. 7, 1925, Stonewall, alt. 8500 ft., near Trinidad, Colorado (H.H. Knight); author's collection. Found on Eriogonum jamesii (Benth.), which may well be the host plant. Allotype: ♀ Aug. 3, 1900, Dolores, Colorado (E.D. Ball).

Phytocoris angustatus new species.

Very slender species that shows some affinities with heidemanni Reuter, in the type of antennae, also having both black and white scalelike pubescence on the dorsum; form of genital segment and the claspers distinctive (Figure 2).

Male. Length 6.1 mm, width 1.83 mm. Head: width 1.08 mm, vertex .30 mm; pale yellowish, vertex fuscous, oblique lines on frons, geminate mark on apex of frons and extending upon base of tylus, transverse mark across middle of tylus, base of juga, and mark on dorsal edge of lora, reddish brown. Rostrum, length 2.65 mm, reaching upon sixth ventral segment, pallid, apical half fuscous. Antennae: segment I, length 1.36 mm, black, having three large, glabrous white spots on dorsal aspect, bearing 8-10 erect white bristles, the black surface clothed with recumbent, black pubescent hairs; II, 3.12 mm, cylindrical, yellowish to fuscous, a narrow white annulus at base; III, 1.63 mm, fuscous, white at base; IV, .95 mm, fuscous. Pronotum: length .85 mm, width

at base 1.56 mm; disk yellowish and marked with fuscous, inner half of calli and central area of disk white; basal edge white, a submarginal fuscous line merging with fuscous area on each side, with two scalloped, tumid black points each side of middle on the submarginal black line, decorated with black scalelike hairs; propleura black, ventral margin and spot at top of coxal cleft, white. Mesoscutum fuscous. Scutellum moderately convex, yellowish, middle of base with fuscous line each side of median line, diverging laterally to reach claval margin, leaving apex broadly yellowish.

Hemelytra with embolar margins straight, subparallel; apical half of corium and the paracuneus, pallid, translucent, inner margin of corium and the clavus except along commissure, dark fuscous, spots on embolium and apex of cuneus also dark fuscous; clothed with a mixture of white and black scalelike hairs, also with simple pubescent hairs. Membrane milky white, sprinkled with dots and patches of color, the larger patches broken by pale dots; veins shaded with fuscous. Legs yellowish white, femora with lines and patches broken by pale dots; veins shaded with fuscous. Legs yellowish white, femora with lines and patches of black on apical half, also with white spots within the black areas; hind femora more broadly black, but posterior and ventral aspects more white than black; front tibiae black, triannulate with white, middle tibiae more broadly white leaving narrower black bands, hind tibiae more broadly black on the basal half. Venter dark fuscous to black, pallid areas on ventral aspect; genital segment and claspers distinctive (Figure 2).

Female. Length 6.4 mm, width 1.9 mm. Head: width .99 mm, vertex .44 mm. Antennae: segment I, length 1.7 mm; II, 3.4 mm; III, 1.77 mm; IV, 1.02 mm. Pronotum: length .88 mm, width at base 1.56 mm. More robust than the male but very similar in color and pubescence, however the white areas more broadly expanded.

Holotype: ♂ Aug. 4, 1917, Prescott, Arizona (H.H. Knight); author's collection. Paratypes: 3 ♂♂, 1 ♀ taken with the type.

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